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PHAGOCYTOSIS
AN EXPERIMENTAL STUDY

With Special Reference to the Opsonic Content
of the Blood.

Being a Thesis Submitted for the
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by

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P R E F A C E .

In the course of this work the following points are considered and in the following order.

	PAGE
Introduction	1.

PART I.

A Review of the Literature on the subject of Opsonins.	5.
A. Their discovery and the nature of the technique employed to demonstrate their presence in the Serum.	6.
B. The nature, mode of action &c. of the opsonic content of Normal Serum.	8.
C. The nature, mode of action &c. of the opsonic content of Immune Serum and a serum the result of a Bacterial Infection.	27.
D. The value of the Opsonic Index in Diagnosis.	34.
E. The value of the Opsonic Index as a control in the Therapeutic inoculation of Bacterial Vaccines.	46.
F. Vaccine treatment of disease.	50.
G. Summary of the conclusions arrived at from a study of the Literature on the subject of Opsonins.	60.

PART II.

Experiments carried out by the writer.	PAGE 64.
A. A description of the technique involved by the various experimental procedures.	65.
B. The variations in the total and differential Leucocyte counts in relation to variations in the Opsonic Indices of Normal Sera.	73.
C. To ascertain whether a serum having a low Opsonic Index for one organism would have an equally low index for other organisms.	77.
D. The variations in the Opsonic Indices of a number of normal healthy individuals.	79.
E. The effect on the opsonic content of keeping serum:- (1.) In contact with the corpuscles. (2.) Separate from the corpuscles.	81.
F. The effect on the opsonic content of keeping serum:- (1.) In an open glass capsule. (2.) In a sealed sterile glass capsule.	85.
G. Effect of incubating partly inactivated serum with fresh washed corpuscles for 24 hours.	87.
H. To ascertain whether a serum inactivated by digestion with one variety of organism would be equally inactivated for all organisms.	90.
I. A few observations made with opsonised Staphylococci.	102.
J./	

- J. To ascertain what effects would be produced on the total and differential leucocyte counts and opsonic indices of guineapigs injected intraperitoneally with Bone marrow and lymphatic Gland Emulsions. 108.
- K. To produce in the guineapig a serum having active lytic properties towards the leucocytes of the rabbit. 112.
- L. To develop in the guineapig a serum having definite immune properties towards Staphylococcus Pyog. Aur. 113.

PART III.

- A. A description of a simple and rapid method of estimating the Phagocytic power of different Bloods. 117.
- B. A number of experiments establishing the accuracy of this method. 122.
- C. The opsonic indices of a number of normal healthy individuals estimated by this procedure. 134.
- D. The tubuculo-opsonic indices of 30 cases of Tuberculosis estimated in the same way. 141.

PART/

PART IV.

	PAGE
A. A general discussion of the experiments recorded in Parts II. & III.	144.
B. The elaboration of a theory as a possible explanation of opsonic action.	186.
C. A summary of the general conclusions arrived at in the course of this research.	211.
D. Bibliography.	223.

I N T R O D U C T I O N .

That the subject of immunity offers a wide field for research, becomes the more apparent the further one investigates this complex question in its various aspects. In fact, so wide is the field presented to the reader and so numerous the channels into which it has been divided by the investigations of more recent years, that it has become a matter of no little difficulty to decide to which of the many aspects of this complex problem one should more particularly devote their attention. In the broadest and most general terms, the subject of immunity from the point of view of research might be said to present the following great problems.

- I. The QUESTION of TOXIN and ANTITOXIN FORMATION.
- II. The QUESTION of BACTERIAL ACTION and the DEVELOPMENT of ANTIBACTERIAL PROPERTIES in the ANIMAL BODY.
- III. The QUESTION of PHAGOCYTOSIS.
- IV. The QUESTION of HAEMOLYSIS and the PRODUCTION of ANTI-HAEMOLYSINS and other allied LYSOGENIC ACTIONS./

ACTIONS.

Of these problems, the first has probably so far met with the most satisfactory explanation and it might also be said, has furnished the starting point of most of the work which has already been done in other fields of immunity. In this connection, Ehrlich's theory has met with such wide-spread acceptance and would seem to explain the various phenomena so satisfactorily that all other theories have become dwarfed in consequence.

With regard to the second problem, namely:- the question of Bacterial action and the production of Anti-Bacterial substances, it cannot be said that the same satisfactory position has been reached. We have here so many factors to deal with that the difficulties become proportionately great, and so the number of theories which have been advanced, to explain the various phenomena individually and collectively, have naturally assumed enormous proportions. In the main the two great theories which might be said to hold the field are, on the one hand, the Cellular theory, supported by Metchinkoff and his fellow-workers and, on the other hand, the Humoral theory, supported by Ehrlich and his followers and, which is, in effect, an adaptation of Ehrlich's original/

original antitoxin theory.

The third great problem presented in the study of immunity is the question of Phagocytosis, more especially in its relation to the Phagocytosis of Bacteria.

The fourth and last great problem, namely:- the question of Haemolysis and other Lysogenic actions cannot be lost sight of on account of the valuable parallel which it forms to Bactericidal actions in general, and much valuable information has already been discovered as a direct result of the study of this analogous process. Amongst the many other problems suggested by a study of immunity, one might perhaps mention, Natural Immunity, also Acquired Immunity, active and passive, their duration and the variations in this duration in the case of different Bacteria, its mode of production and the cause of its persistence in some cases and not in others and also the question of recovery from Bacterial Infection. Every one of these problems might be said to require further investigation as none of them would seem to have so far met with an entirely satisfactory explanation. Nor are these, by any means, the only problems requiring investigation for, on each of these are dependent innumerable side issues, all of which call/

call for careful study and research.

From all these problems the question of Phagocytosis has been selected for special study and to it attention has been entirely confined in this work, except in so far as a study of the other problems has helped to elucidate many of the phenomena observed in the case of the former.

In regard to the question of Phagocytosis, attention has been more especially directed to a study of that particular constituent of the Blood, comparatively recently discovered by Sir A.E.Wright and designated by him opsonin.

In concluding this brief introduction, I should like to express my indebtedness to Professor Greenfield for the privilege of carrying out this research in the Pathology Department of the University. I have also to acknowledge a grant from the Earl of Moray fund in aid of this work.

PART I.

Which is entirely devoted to a discussion of the Literature relating to the opsonic content of the blood, its possible origin, nature and mode of action, etc.

THE OPSONIC CONTENT OF THE BLOOD.

The experiments which directly led up to the discovery of opsonins were first described by Leishman, and were devised by him to ascertain if any difference could be demonstrated in the phagocytic activity of the Blood of an individual suffering from a persistent Staphylococcal Infection as compared with the blood of a normal healthy man.

The technique employed consisted in mixing on a slide a definite quantity of the Patient's blood with a definite quantity of an Emulsion of Staphylococcus Pyogenes Aureus, covering this with a cover glass and placing the slide in the incubator at 37°C. for a short time. The cover glass was then separated and the resulting film preparation fixed and stained in the ordinary way. The number of Bacteria ingested by a definite number of the polymorph leucocytes was then estimated, the leucocytes being counted in sequence. A similar procedure was carried out in the case of a normal blood, usually on the same slide, and the resulting phagocytosis similarly estimated, compared with that obtained in the case of the abnormal blood. In this way a fairly accurate comparison/

comparison of the phagocytic activity of the two bloods was obtained and as a result Leishman satisfied himself that the phagocytic activity of the blood of a patient suffering from a persistent Staphylococcal infection was distinctly less than that of a normal individual, and furthermore that the inoculation in such cases of a Staphylococcal Vaccine resulted in an increased phagocytic activity. Working on the lines suggested by Leishman's observations Wright next introduces a modification of this technique, whereby the actions of the corpuscular and fluid elements of the blood could be separately studied. In short the technique is as follows: -

1. Leucocytes are obtained by centrifugalisation of a few drops of the blood of a normal individual run directly into a saline solution containing a percentage of Sod. Citrate and subsequently freed from the fluid elements by washing in normal saline solution.
2. Serum is obtained in the ordinary way by allowing the blood to clot.
3. Bacteria are suspended in saline solution thus forming the Emulsion.

In this way it was possible to study the phagocytic/

phagocytic activity of the leucocytes either in conjunction with or apart from the Blood serum. In estimating the phagocytic activity of the blood definite quantities of these three ingredients are accurately measured by means of a specially graduated pipette, thoroughly mixed and the resulting mixture incubated in a sealed capillary for a definite time. A film preparation is then made, fixed and stained and the average number of Bacteria ingested per leucocyte estimated. When two bloods are compared the same leucocytes, for reasons which will appear later, may be employed with each serum. The average number of ingested Bacteria per leucocyte is estimated in each case and the ratio of these values taken as the result or "Index". This technique is described in detail in another place - vide infra - and it will therefore be unnecessary to make further reference to it here.

As a result of a large number of experiments conducted with this technique by Wright and Douglas, the following conclusions were arrived at by these authors: - That phagocytosis is due to the presence, in the blood fluids, of a substance, which acts on the Bacteria preparing them for phagocytosis. That the Bacteria are incapable of being phagocyted unless so/

so prepared, that this substance is present in the serum of all normal healthy individuals, that it is almost, if not entirely, destroyed by heating to 60°C. for 15 mins. and that to a large extent it is lost on standing. To this substance the name opsonin has been given by these authors. In a subsequent investigation Wright and Douglas studied this opsonic action of serum with a great many different varieties of Bacteria and it was found that the same opsonic action could be demonstrated in the case of all the Bacteria examined with the exception of the Diphtheria Bacillus and the Bacillus Xerosis. They were also able to show that the leucocytes employed in estimating the degree of phagocytosis of different bloods, were quite an indifferent factor, the result being entirely dependent on the opsonic content of the Blood serum.

Working on parallel lines, Bulloch and Aitken employing the technique of Wright, conducted a large number of exhaustive experiments, the results of which they summarise as follows: -

1. Opsonin is present in Normal serum.
2. It is thermolabile.
3. It rapidly disappears from the serum when the latter/

latter is mixed with Bacteria at 37°C. and at 0°C.

4. After the opsonin has united with the Bacteria the mixture of serum and cocci can be heated to 60°C. for long periods without abolition of the opsonic effect.
5. The leucocyte is practically an indifferent factor when the phagocytic power of different bloods is compared.
6. The capacity of Bacterial Emulsions for extracting opsonin from the serum is only slightly diminished by subjecting these Emulsions to very high temperatures over prolonged periods.
7. The action of heat is to destroy the opsonin and not merely to convert it into a nonopsonisable modification.
8. The opsonin is not identical with any of the antibodies hitherto discovered in the serum.
9. Opsonin is of a relatively simple constitution.
10. When these experiments cover the same ground as those of Wright and Douglas, the observations of these authors are confirmed."

As a result of still further investigation Wright and Douglas have confirmed their previous observations and in addition have also been able to demonstrate, /

demonstrate, that the blood of an infant at birth contains opsonin in almost the same proportion as the maternal blood, and that the tissue serum obtained from a blister raised by friction contains opsonin in almost the same proportion as the Blood serum. Wright, in a further article, calls attention to the question of spontaneous phagocytosis. In this connection he points out, that in mixtures whose salt content falls below 1% a considerable degree of phagocytosis is observed, even in the complete absence of any serum, which phagocytosis he terms spontaneous phagocytosis. On the other hand, if the salt content of the mixture is kept above 1% this spontaneous phagocytosis is abolished. For all practical purposes, however, as Wright himself points out, this fallacy may be entirely overlooked. In the same communication attention is also drawn to the necessity of heating sera for the same length of time and at the same temperature when a comparison of the results is desired. The general results of Wright's work on the opsonic content of normal serum have in most cases been confirmed by various other observers, and in this country have been very generally accepted. In so far, however, as certain objections have been raised, it will be necessary to refer to them here, and/

and of these the two which perhaps call for special mention have been put forward, on the one hand by Dean and on the other hand by several American authors. Dean's objection has reference to the question of the thermolability of the opsonin in normal serum, and is to this effect: - That the fall in the opsonic content of normal serum observed by Wright and others does not necessarily imply a total destruction of the opsonins, but merely indicates that they have been reduced to a concentration below that demonstrable with the technique employed by Wright and Douglas. Where Dean employed the technique of Wright and Douglas his results entirely corresponded with theirs, on the other hand, where a different technique was used, the results thus obtained were entirely at variance with the former. In short the technique employed by Dean was as follows: -

Bacteria were digested with a large quantity of heated normal serum, separated from this by centrifugalisation, and washed free from any adhering serum in saline solution separated from this by centrifugalisation and subsequently tested with washed corpuscles alone, to ascertain if they had been opsonised by digestion with the heated serum. A control was furnished by testing in the same way Bacteria/

Bacteria which had been (a) Digested with unheated serum, and (b) which had not been previously digested at all. As a result of numerous experiments carried out in this manner, Dean concludes, - that the substance in normal serum designated opsonin by Wright, is not a new body, but is in reality identical with the "Fixateur" or "la substance Sensibilisatrice" of the French School: that, although a certain fall in the phagocytic power is observed on heating, it is thermostable for several hours at 60°C.: that the same substance is found in immune serum in greater bulk. Finally Dean concludes with the statement, that as the term "Fixateur" or "Substance Sensibilisatrice" is used to designate many other properties of immune serum, one should therefore perhaps adopt the term opsonin for that particular property which it possesses of preparing Bacteria for phagocytosis. He also suggests that the process of phagocytosis may be analogous to Bacteriolytic and haemolytic actions, and due to the action of an immune body plus a complement, and that the slight fall of phagocytic power on heating may be due to loss of complement.

This suggestion in itself is of no little interest as it is one of the few recorded by which an/

an attempt has been made to bring the question of phagocytosis into line with what is known of other Bactericidal processes. With regard to the thermolability of the opsonin of normal serum, the question according to Dean would seem to depend on the insufficient nature of the technique employed. It is not perhaps clear why the technique employed by Dean should be the more accurate of the two, and a perusal of the results obtained thereby does not impress one in its favour. Thus in many of the experiments the average number of ingested Bacteria per leucocyte is very high . as many as 50 per leucocyte in some cases a figure which is admittedly considerably beyond the limit of accurate enumeration. Then again, it is stated that the Bacteria were digested with a large quantity of heated serum, but the exact amount is not specified, nor is the time to which it was subjected to heat stated, nor the form of receptacle in which the process of heating was conducted, all of which points would materially influence the results. For these reasons, and taking into consideration the very large weight of evidence on the other side, the thermolability of the opsonins of Normal Serum must, I think, be accepted until more conclusive proof can be brought forward in favour of the contrary opinion.

The/

The second objection which we must consider has been advanced by Simon, Lamar and Bispham, who conjointly in an elaborate article recently published offer considerable criticism to some of the results established by Wright and others. As in the case of the objection raised by Dean, these authors also in the first place criticise the technique employed by Wright on the grounds that it does not give an adequate idea of the actual quantity of opsonin present in the serum; that the average number of ingested Bacteria per leucocyte may be so great that enumeration is out of the question: that with certain organisms, spherulation and loss of staining power, commonly occur and frequently preclude all possibility of obtaining a satisfactory result by counting: and that the agglutinating effect of certain sera on the corresponding organisms is a further drawback. Led by these considerations, they claim to have devised a new method more satisfactory and free from the objections which attach to Wright's technique. On analysis the two main differences in this new method are found to be: -

1. Instead of the pure undiluted serum, dilutions of 1 in 20, 1 in 30, and 1 in 40 are employed.
2. Instead of estimating the average number of ingested Bacteria per leucocyte, only the percentage/

percentage of phagocytizing leucocytes in the case of each dilution is estimated.

In other respects the two methods are practically identical as far as the general principles are concerned, but differing in one or two of the details. Thus for example in the case of Wright's Method, a definite quantity of the same Bacterial Emulsion is added to the various mixtures of Leucocytes and serum. In the other method, however, it is stated that each sample of the diluted serum is inoculated with a small quantity of the organism, and no indication is given of any steps having been taken to ensure that the resulting emulsions in different cases were even approximately of the same density; we must therefore, I think, conclude that in the different mixtures incubated, Bacteria must have been present in greatly varying degrees of concentration.

Then again, in the case of Wright's method, the phagocytic power of an abnormal (or normal, as the case may be,) blood is in every case compared with the phagocytic power of a normal, control blood estimated with exactly the same Bacterial Emulsion, and the Index expressed in the final result and known as the Opsonic Index, is in effect the ratio which the phagocytic power of the abnormal blood bears to that of the Control blood.

On/

On the other hand with the Method of Simon Lamar and Bispham, no such comparative control was carried out, but each serum, so to speak, was tested on its own merits alone.

Taken together these two defects must influence the results to a very great extent, and especially must this be the case where the phagocytic power of different sera is compared.

It is therefore not surprising that these authors have found a very much wider range of variation in the opsonic indices of normal individuals, than other observers who have employed what one must consider, for the reasons already stated, the more accurate method of Wright. A consideration of some of the results obtained by Simon, Lamar and Bispham shows in a striking manner the variable character of these. Thus for example, in a number of estimations of the opsonic content of the blood of normal healthy individuals, the following variations were obtained:-

With 1 in 20 dilutions of the serum the percentage of phagocytizing leucocytes varied from 12% to 100%; with 1 in 30 dilutions, from 2% to 100%; with 1 in 40 dilutions, from 0% to 100%.

The extraordinary wide range of variation is at once manifest, and needs no further comment. Other results obtained by these authors are as follows:-

The opsonic content is apparently affected to a considerable extent by the ingestion of food, an increase/

increase being commonly noted after meals. The opsonic content of the Blood of nursing infants is higher than that of the normal adult, and this high value is, they think, referable to the fact that the nursing infant is practically in a state of continuous digestion.

That the opsonins are essentially components of the blood, and in all probability formed there. That no parallel can be demonstrated to exist between the opsonic content of the blood, and either the total number of Leucocytes or any one variety of leucocyte. With regard to the chemical nature of opsonins they conclude that "the opsonins even though themselves not necessarily of the nature of globulins, are nevertheless intimately associated with these as in the case of the various antibodies as shown by Pick. (Pick E. Hofmeister's Beitrage 1901 - 1. 351.)"

They have also come to the conclusion as a result of various absorption experiments that the opsonins of normal serum are in no way specific, but that the opsonins may be a constant quantity, and that the number of organisms which are taken up by a cell is influenced by a second factor which may be variable.

The/

The question of the specificity of the opsonins of normal serum, suggested by this last conclusion is one which must now be considered. With regard to the opsonins of immune sera, there seems little doubt that a high degree of specificity exists and this had been clearly demonstrated by many observers, but in the case of normal serum, with the exception of the above communication and one by Bulloch and Western, almost no reference has been made to this question of specificity in any of the published memoirs on the subject of opsonins. Bulloch and Western, however, have published conjointly a number of experiments in this connection as a result of which they conclude that even in normal serum a high degree of specificity exists for different organisms.

The following results were obtained by these authors: -

1. When Staphylococci are brought in contact with normal human serum, and are subsequently removed by centrifugalisation, the serum loses its opsonic power for Staphylococcus although the opsonic power for B. Pyocyanus is preserved.
2. When T.B. are brought in contact with normal human serum and are subsequently removed by centrifugalisation the serum loses its opsonic power for T.B., although the opsonic power for Staphylococcus/

Staphylococcus is preserved.

3. Similarly where Staphylococci are brought in contact with normal human serum and subsequently removed, the serum loses its opsonic power for Staphylococcus while retaining it for T.B.
4. Inoculation of a normal being with new Tuberculin causes a quantitative increase in Tuberculo-opsonins, whereas the quantity of Staphylococcal opsonins is unaffected.
5. Inoculation of a normal being with Staphylococcal Vaccine causes a quantitative increase in the Staphylococcal opsonins, whereas the Tuberculo-opsonins remain unaffected.

These results are very interesting, and entirely opposed to those of Simon, Lamar and Bispham. It may be mentioned in passing, that a large number of similar experiments were carried out by the writer on the assumption that this question had not been previously investigated, the results of which in a large measure confirm those of Bulloch and Western.

From time to time various interesting and instructive additions have been made to the literature of opsonins. Many of these have been simply confirmatory of previously established facts, but on the other hand some have been on entirely new lines, and/

and as a result new ground has been opened up.

Of the more important of these might be mentioned a recent communication by Professor Muir in conjunction with Martin, dealing with the possible composition of the opsonic content of normal serum. To put it shortly, the question which these authors set themselves to solve was, whether the opsonins of normal serum could be shown to behave in a manner in any way corresponding with the behaviour of certain already well-known complements. In this connection a series of experiments were carried out to ascertain what effects on the opsonic power of normal serum would be produced by certain methods of absorption already known to lead to the fixation of Haemolytic and Bacteriolytic complements. For this purpose the immune bodies corresponding to the receptors of red blood corpuscles, Blood Serum and Bacteria, were employed. Thus it was already well known that red blood corpuscles sensitized with their corresponding immune body were capable of removing entirely from normal serum, not only the complement concerned in haemolysis, but in addition the complement concerned in Bacteriolysis; would the opsonins in normal serum be removed in the same way?

As a result of their experiments Muir and Martin/

Martin were left no room to doubt that opsonins were completely removed by a similar process of absorption. These authors also showed conclusively that, in addition, serum receptors sensitized with their corresponding immune body and also Bacteria sensitized with their immune body, each in their turn were capable of entirely removing the opsonins from normal serum. They therefore conclude that the opsonins of normal serum are certainly of the nature of complements.

In the experiments conducted by Muir and Martin an emulsion of *Staphylococcus Pyogenes Aur.* was employed in testing the opsonic power of the serum and *B.Coli* sensitized with appropriate immune body were used in the absorption experiments to ascertain whether this combination in addition to removing haemolytic complement from normal serum would also remove the opsonins. In connection with this experiment it is interesting to note that whereas *B.Coli* plus Immune body practically entirely removed the opsonins from normal serum, when the latter was subsequently tested with *Staphylococcus*, on the other hand, in a control experiment in which *B. Coli* were used alone, i.e., where no immune body was added, only about 40% of the opsonins were removed from normal/

normal serum when this was subsequently tested with *Staphylococcus*. This it will be seen bears out what has already been said in regard to the specificity of opsonins in Normal Serum.

Other experiments, which might be mentioned on somewhat parallel lines have been recorded by Keith. Instead, however, of Bacteria being employed to test the phagocytic power of serum, Red Blood Corpuscles were used and the question which Keith set himself to solve was not, as in the case of Muir and Martin, whether opsonins were of the nature of complements, but whether they, i.e., the opsonins, were identical with the amboceptors, i.e., immune bodies concerned in haemolysis. It is not proposed to give these experiments in detail, it will suffice if the general conclusions arrived at by Keith are quoted: they are as follows: -

That the phagocytosis of Red Blood Corpuscles does not depend on the presence of the haemolytic amboceptor since: -

1. The substance which induces phagocytosis of Red Blood Corpuscles is practically destroyed by heat while the haemolytic amboceptor is entirely thermostable.

2. The haemolytic amboceptor may be present in/

in considerable amount in a haemolytic serum without inducing phagocytosis, notwithstanding prolonged contact of amboceptor with the Red Blood Corpuscles. Keith therefore concludes that the phenomenon of phagocytosis is caused by some special body belonging to the class of opsonins. Taken together the observations on the one hand of Muir and Martin, and on the other of Keith, are of extreme interest as opening up a new line of investigation which may ultimately lead to the action of opsonins being brought definitely in line with what is already known of other more or less analogous processes. Amongst other points which have been investigated in connection with opsonins, one might perhaps mention the various observations which have been carried out to ascertain how far the presence of opsonins could be demonstrated in the various secretions of the body. That opsonins are present in many has been amply proved, and of these perhaps the most interesting, from the standpoint of practical medicine, is milk. In this connection, reference might be made to a communication of no little interest by Professor Sims-Woodhead, and Mitchell. These observers examined the opsonic content of the blood and milk of healthy normal cows, and also of cows which were either known definitely to be suffering from Tuberculosis or in which/

which the presence of this condition was suspected. Their results are of some interest in that the milk of Tubercular and also unhealthy cows invariably showed a low tuberculo-opsonic index, whilst the milk of healthy cows invariably showed a high index, in some cases an index of 2.2 being recorded. Pursuing these investigations further, they found that milk whey produced with Rennet or hydrochloric acid contained a larger proportion of opsonins than the total milk. They also demonstrated that T.B. after digestion with whey, were actively opsonised. That the question opened up by these observations is of very considerable interest is at once manifest, for, as the authors suggest, it is quite within the bounds of possibility that a milk rich in tuberculo-opsonic elements may be capable of conferring upon the individual partaking of it, by absorption from the digestive tract, a certain degree of passive immunity to the Tubercle Bacillus. Should this suggestion be proved correct in light of adequate experimentation, then a very decided advance will have been made in connection with the prophylaxis of Tuberculosis and in fact, its utility may prove to have a much more extensive application than in the mere prophylaxis of the disease.

Another observation which might be referred to as having some bearing on the possible source of opsonins/

opsonins in normal serum is contained in a recent communication by Capps and Smith. These authors in an investigation carried out in connection with the therapeutic application of X Rays in cases of Leukaemia make the following observations: -

1. X Ray produces in Leukaemia a disintegration of the leucocytes, especially the young forms, viz. the myelocytes and nongranular mononuclear cells.
2. Serum of a Leukaemic patient who has improved under X Ray treatment causes Leukopenia when injected into animals, and when added in the hanging drop to the leucocytes of another individual it disintegrates the mononuclear cells.
3. In spite of this disintegration of the cells, no material alteration of the phagocytic power of a blood so treated, in vitro, can be demonstrated.

This last observation is of some little interest as in some measure eliminating at least the mononuclear variety of leucocytes as a possible source of opsonic production. The only other question in connection with the opsonic content of normal serum which remains to be discussed, is in regard to the range of variation in normal individuals, but, as this has been entered into fully in another place; vide infra, no further reference need be made to it here./

here. So much for the opsonic content of normal Serum; from which we must now turn our attention to a consideration of the opsonic content of the Serum in cases where a condition of immunity has been established either artificially by appropriate inoculations or as the result of an attack of the specific disease produced by a definite organismal infection.

In speaking of all the sera included in this definition as immune sera, one would seem to be applying the term in a somewhat elastic sense. True the inoculation of normal animals with definite Bacterial vaccines in most cases results in the production of a serum having definitely immune properties, and so also in man, some diseases, such as Typhoid, Malta Fever and Pneumonia and some others, all of which run a comparatively acute course culminating in recovery, produce a serum also having definitely immune characteristics, but on the other hand, several of the more chronic forms of Bacterial infection, such as tubercle and some varieties of Staphylococcal infection would rather seem to result in the production of a serum having considerably less resisting power than the normal, and to these the term immune seems hardly applicable. However, in so far as these sera, though their power of resistance may be considerably/

considerably diminished, can, in most cases be shown to contain certain elements not present in normal, but only as a rule. in immune sera, the term immune may have in reality more justification than at first sight appears. With regard to the opsonic content of such sera, many facts have been elucidated, some of which have been almost universally accepted, in respect of others, however, a good deal of controversy and diversity of opinion would seem to exist. In the main its mode of action would seem to be entirely analogous to what has been generally accepted as being the mode of action of the opsonins of normal serum, and as a result, most authorities, at least in this country, have accepted the term "opsonic content of immune serum" as its designation. Apart from its mode of action, however, it differs very materially in some respects from the opsonins of normal serum. Thus, it is generally admitted to be thermostable, resisting a temperature of $60^{\circ} - 65^{\circ} \text{C.}$ for prolonged periods. In this connection, however, Wright though one of the first to point out this property, has subsequently thrown doubt on it, and in fact, the position which he would latterly seem to have taken up is, that the opsonin of immune serum in no way differs from the thermolabile opsonin of normal serum, but can/

can be readily destroyed at a temperature of 60° C. The experiment on which this opinion is based consisted in diluting immune serum prior to heating, when it was found that, provided adequate dilution was carried out prior to heating, immune serum so treated gave up its opsonin more readily with progressive dilutions, than an immune serum which was similarly diluted but not heated; and further, that the diluted and heated serum was entirely inactivated when a dilution was reached at which the similarly diluted but unheated serum still showed a very considerable phagocytic power. From this experiment, Wright concludes that the opsonin in immune serum which is found after heating to 60° C. is nothing more than a residue of the original opsonic content, which has escaped destruction by the heat applied, and that the opsonins found in normal and immune sera are identical.

Whether Wright is really justified in considering the opsonins in immune serum as thermolabile in light of this dilution experiment has led to no little controversy, and as a result, most authorities would seem to have taken up the reverse position, and it is now almost generally held that, at least, part of the opsonic content of immune serum is definitely thermostable. This leads us to another point on which some/

some diversity of opinion also exists, namely:- whether this thermostable element in immune serum is in reality an opsonin at all, i.e. in the meaning of the term as defined by Wright. In the first instance, Wright considered that it was definitely a thermostable opsonin, subsequently, however, in light of the above experiment considering it a thermolabile opsonin. Dean, on the other hand, while agreeing with Wright that the elements in normal and immune sera are the same, maintains that they are definitely thermostable in both cases, and further that they are identical with the "Fixateur" or "Substance Sensibilisatrice" of the French School.

Then again, Leishman takes up quite a different position, holding that the thermostable element in immune serum is in no way akin to the thermolabile opsonin of normal serum, but that it is an entirely different substance, and further that its action is not that of combining with the Bacteria, thus preparing them for phagocytosis, but that it has a definite stimulative action on the leucocytes themselves, and to this substance he applies the term "Stimulins".

It will thus be evident that the exact nature of the opsonic content of immune serum is a matter/

matter of some doubt, but taking into consideration the facts already stated, and in addition the evidence brought forward from many other sources, the weight of evidence would seem to point to the opsonic content of immune serum consisting of two elements, one which, like the opsonin of normal serum, is thermolabile, the other differing from this in that it is definitely thermostable, and further that both these elements act in a manner apparently identical to the opsonins of normal serum, by preparing the Bacteria for ingestion by the leucocytes. With the lapse of time, controversy on this question has somewhat subsided, and at the present time the position as above defined would seem to have been tacitly accepted by general consent.

Another characteristic of the opsonic content of immune serum must now be referred to, namely, that it is in a high degree specific, only acting - (the thermolabile elements having been destroyed by heating to $60^{\circ}\text{C}.$) - on that organism for which the condition of immunity has been established. It will thus be seen that the only salient difference between the opsonins of normal and immune serum resolves itself into a question of how far the latter are thermostable. Other minor differences have perhaps been recorded/

recorded; thus, for example, Wright in one of his earlier papers, called attention to the fact that the reputedly thermostable opsonin of immune serum is in a high degree heliolabile, being destroyed entirely by exposure to direct sunlight for a period of 6 to 8 hours. That this is contrary to what is generally found to hold in the case of thermostable bodies in general, he brings forward as an additional argument in favour of his contention that the opsonins in immune sera are in reality thermolabile. A general consideration of these points would rather seem to confirm one in the impression that immune serum must contain two more or less distinct elements: -

1. Eminently thermolabile but comparatively heliostable.
2. Eminently heliolabile but comparatively, if not entirely, thermostable.

It would thus seem that, as a result of the production of a condition of immunity, the opsonins already present in the normal serum are, in the first place, increased, and that in the second place, another element having certain definite and distinct characteristics is developed, this latter element gradually increasing at the expense of the former. Whether/

Whether or not this opinion is actually held by the various authorities, individually, it is difficult to be certain, as no definite statements have been made in this connection in more recent literature, but it is, I think, the conclusion which one is forced to accept in light of the various conflicting statements on record.

In regard to the source of the opsonic content of immune serum, little or no evidence is forthcoming, observation having been almost entirely confined to an investigation of the opsonic content of such sera under abnormal conditions.

This brings us to a consideration of perhaps the most important question raised by the discovery of opsonins, namely, the adaptation of this discovery to practical medicine. In this connection there are three important questions which it would perhaps be better to consider separately, and in the order named; they are:-

- I. Is the OPSONIC INDEX of any REAL PRACTICAL VALUE in the DIAGNOSIS of DISEASE?
- II. Has the OPSONIC INDEX any PRACTICAL APPLICATION in THERAPEUTICS?
- III. Is the VACCINE TREATMENT of DISEASE JUSTIFIED by the RESULTS ALREADY OBTAINED?

I. IS THE OPSONIC INDEX OF ANY REAL PRACTICAL VALUE IN THE DIAGNOSIS OF DISEASE?

A satisfactory answer to this question can only be arrived at by a consideration of at least three separate points. In the first place, we have to consider what are the normal limits of variation of the Opsonic Indices in healthy individuals and for different organisms. Secondly, we must consider the limits of variation in the case of patients suffering from a known disease for the organism producing that disease, and also for other organisms. And thirdly, in the present case, we have to consider whether the Opsonic Index per se can be in any way supplemented in order that the results obtained thereby may be of greater practical value.

In connection with the diagnostic value of the Opsonic Index, by far the greatest amount of evidence has been accumulated in relation to the Tubercle Bacillus, and for this reason it is proposed to confine our attention in the first instance to the Tuberculo-opsonic Index, subsequently referring to the behaviour of the opsonic index, in relation to some/

some other varieties of Bacterial Infection.

It is not proposed to give in detail all the observations, which have from time to time been recorded in relation to the limits of variation of the Tuberculo-opsonic index in health; it will suffice for our purpose if it be simply stated that a very large number of such observations have been forthcoming from a great variety of sources, as a result of which it has been definitely established, that the tuberculo-opsonic index in health may vary from .80 to 1.20.

If we now turn our attention to the Tuberculo-opsonic index in cases of undoubted Tuberculous Infection, we at once find that a very considerable proportion of these fall within the normal limits of variation. In all, between 400 and 500 estimations of the tuberculo-opsonic index, in cases of undoubted Tuberculosis, have been collected from the general literature, recorded by Wright, Bulloch, Urwick, Lawson and Stewart, Ross and others, and of these about 25% fall within normal limits. Of the remaining 75%, a very large proportion, in Urwick's cases 55%, and in Ross's 51%, show an index above 1.2; in the remaining 20 or 25%, a low index was recorded. There is thus ample evidence that the tuberculo-opsonic index of patients suffering from Tuberculosis/

Tuberculosis varies within very wide limits, and taking into consideration the fact that at least 25% of these indices fall within the limits of normal variation, it is at once evident that the Tuberculo-opsonic index, per se, can in no way be relied on in the diagnosis of Tuberculous Disease. When a low or high index is obtained, the evidence is certainly strongly in favour of Tubercle, but on the other hand where a normal index is obtained, it is quite impossible to definitely exclude the presence of Tuberculous disease.

Of this wide range of variation in the Tuberculo-opsonic indices of patients suffering from Tuberculosis, Wright brings forward a lucid and, at the same time apparently a highly satisfactory explanation, based on the observance of two striking facts, namely:-

1. The Tuberculo-opsonic index of certain cases of Tuberculous infection varies, in one and the same case, within wide limits from day to day, in some cases from far below normal, to as much as twice normal or even higher.
2. That the effect of inoculating a Tubercle Vaccine is, in the first instance to cause a distinct fall in the Tuberculo-opsonic index, the negative phase./

phase, and that this subsequently gives place to a distinct rise - the positive phase.

Taking these two facts together, Wright comes to the conclusion, that in a Tuberculous case showing a distinct variation in the Tuberculo-opsonic index from day to day, some of the Tuberculous material is from time to time cast off from the seat of infection into the general circulation, and that this acts in exactly the same way as the inoculation of a Tubercle Vaccine, the low indices thus corresponding to a negative phase, the high indices to a positive phase. The daily fluctuations in the Tuberculo-opsonic indices of some cases of Tuberculosis thus resolve themselves into a succession of positive and negative phases the result of an auto-inoculation of the general system from the focus of infection. In regard to the value or otherwise of the Tuberculo-opsonic Index per se, in the diagnosis of Tuberculous disease, Wright takes up the following position:-

That Tuberculous Infections as a whole are divisible into two distinct classes.

- I. Cases where the lesion is strictly localised and unaccompanied by any constitutional disturbance
- II. Cases where the symptoms are more acute and cases of/

of generalised tuberculosis, accompanied by general constitutional disturbance.

In the first class, a persistently low index is almost invariably met with: in the second class, the index fluctuates greatly from day to day. Thus, where several estimations of the Tuberculo-opsonic index can be obtained:-

- (a) A persistently normal index excludes tubercle.
- (b) A persistently low index indicates a localised tuberculous lesion.
- (c) A daily fluctuation in the index indicates an active acute infection, or a generalised Tuberculosis, accompanied by general constitutional disturbance.

When only one estimation of the Tuberculo-opsonic index is procurable:-

- (a) A normal index indicates nothing - Tubercle cannot be excluded.
- (b) A low index indicates either a localised lesion, or an acute infection depending on the presence or absence of constitutional disturbance.
- (c) A high index indicates active Tubercle with/

with constitutional disturbance.

These propositions enunciated by Wright have been confirmed by many other observers and they sum up practically all that can be said regarding the value of the tuberculo-opsonic index in the diagnosis of tuberculous infection, at the same time, perhaps, putting the question in its most favourable light. It will thus be seen that the tuberculo-opsonic index per se where only a single estimation is procurable can, at least, only be regarded as a somewhat doubtful aid to diagnosis with the exception of those cases in which a definitely low or a definitely high index is obtained. Where several estimations are procurable its value is very materially increased, but this again brings us to another point which cannot be lost sight of in discussing the practical value of a method of diagnosis, and that is the laborious and complicated nature of the technique involved, and in which a very considerable degree of precision is necessary to ensure accurate results. Taking into account all these considerations it is hardly to be expected that a method, so laborious and, at the same time by no means of definite diagnostic value, should recommend itself for practical daily use.

That being so, it seems hardly necessary to/

to discuss the remaining question, namely whether it is possible to supplement the opsonic index, in any way that will increase its practical value, as this can only result in making an already complicated process even more complicated. However, as various methods have been suggested they had perhaps better be mentioned briefly. Wright is again responsible for most of the suggestions in this direction, and of these the following three will be referred to.

1. In active systemic tubercle the serum when heated to 60° C. still retains the power of exciting phagocytosis to a greater or lesser extent.
2. By a comparison of the opsonic index of the supernatant fluid obtained by centrifugalisation from the pus or exudate obtained from the seat of infection, with the opsonic index of the blood.
3. If Koch's New Tuberculin (T.R.) is inoculated in the case of an individual suffering from a tuberculous infection, the negative phase is much more readily induced, is more marked and persists longer than in the case of an individual not the subject of a Tuberculous Infection.

It is not proposed to discuss the first two methods as they have at best only a limited range of application, and the results so far recorded are too few/

few and not of a sufficiently consistent nature to recommend their general adoption.

In the third method, however, there can be little doubt that we have a very delicate test for the presence or absence of Tuberculous Infection; there is already more than sufficient evidence from various sources to justify such an assertion, but here again one is confronted with the laborious nature of the procedure as in this case not only must an estimation of the Tuberculo-opsonic index be made prior to inoculation, but daily estimations must be made for several days thereafter.

To sum up the whole question, one is forced to the conclusion that the technique involved is so laborious, and the results so uncertain that the general practical utility of the various procedures suggested, is thereby in a large measure detracted from.

What has already been said in regard to the diagnostic value of the opsonic index in disease refers more particularly to Tuberculous Infection, but the same to a greater or lesser extent is applicable in the case of Infections resulting from many other varieties of Bacteria. Of the other Bacterial Infections which have been studied in this connection, reference/

reference might perhaps be made briefly to those, the result of invasion by:-

Staphylococcus, Pneumococcus, Gonococcus, Micrococcus Melitensis, Bacillus Typhosis, and Bacillus Coli.

In connection with Staphylococcal Infections a great deal of evidence has been accumulated and, as a result it would seem that the opsonic index per se, is here of very considerable diagnostic value. Here at all events a consistently low opsonic index is the rule, and there is no evidence of high indices corresponding to those obtained in the case of Tuberculous infection, and resulting from auto-inoculation from the seat of infection. One must not forget, however, that by far the greater number of observations in this connection have been made in cases in which the skin has been the seat of Infection, and which might very properly be taken as corresponding to one variety of Tuberculous infection, namely:-

Lupus, a condition in which a low Tuberculo opsonic index is also very frequently obtained. Before taking up any definite position in regard to the diagnostic value of the Staphylococcal-opsonic index, one would naturally like to know more about its behaviour in cases of infection characterised by marked constitutional disturbance as, for example, cases/

cases of acute Osteomyelitis, acute middle ear disease, and other varieties of more deep-seated infection. Up to the present, few if any results have been recorded in such cases, and naturally, of course in this connection its value even were it proved to behave in an uniform manner is very limited, as the condition invariably calls for prompt operative interference, and it is of little importance what the exact nature of the infection may be. Still as a process analogous to Tuberculous infections accompanied by constitutional disturbance, the behaviour of the Staphylococcal-opsonic index in such cases would be highly instructive.

In cases of acute lobar pneumonia, it has been found by MacDonald that at the commencement of the infection, and whilst the temperature is rising and continuing throughout the fastigium, the pneumo-opsonic index is low; with the advent of the crisis it rises and continues to do so, eventually reaching a value considerably above the normal as high as 1.6 in some cases. From this it would seem that the pneumo-opsonic index is of some diagnostic value, but the observations so far recorded are too few to permit of any definite statement being made.

Likewise in the case of Gonorrhoeal infections/

infections, the observations recorded are too limited to enable one to speak with certainty, but from observations which are on record, it would appear that the Gono-opsonic index varies within very wide limits, corresponding in this respect to the tuberculo-opsonic index.

In the case of the Gonococcus some accurate method of diagnosis would be of the very greatest practical value, on account of its wide range of application. Thus, for example its value must be at once manifest not only in the department of the Gynecologist, when at best the diagnosis of gonorrhoeal infection can in many cases only be arrived at by a process of exclusion, but also in General Medicine and Surgery in the diagnosis of gonorrhoeal rheumatism, and other infections of a more or less surgical nature.

In regard to the opsonic index in Typhoid Fever and Malta Fever, a considerable amount of work has been done especially by Leishman, and as a result of these observations it would appear that the corresponding index in these infections is considerably increased. In the case of Bacillus Coli, a low index is frequently obtained in conditions of infection by this organism, but further investigation is necessary/

necessary before its true diagnostic value can be definitely appreciated.

Not the least interesting of the observations recorded regarding the behaviour of the opsonic index in disease are those of Jacobs and Geets in connection with *Micrococcus Neoformans* in cases of cancer. In a series of cases of cancerous growth these observers have recorded a low opsonic index for this organism, and they have also demonstrated in many cases a very considerable rise in this index, as a result of treatment by inoculation of a vaccine prepared from a culture growth of the organism, a corresponding diminution in the size of the cancerous growth being observed at the same time. Wright also records one or two satisfactory results following upon treatment of cancerous patients on similar lines but here again as in the case of most of the other organismal infections to which reference has been made, much fuller investigation is yet required.

So much for the diagnostic value of the opsonic index in disease, the whole question might be conveniently summed up in the words so often repeated already - further investigation -. Until the results of more extended observation are forthcoming this question must, I think be left an entirely open one. We shall now turn to a consideration of the second/

second question which claims our attention - namely:-

II. HAS THE OPSONIC INDEX ANY PRACTICAL APPLICATION IN THERAPEUTICS?

Here again by far the greatest amount of evidence has been accumulated in connection with the Tubercle Bacillus, but in this case the behaviour of the opsonic index is practically identical for all organisms, and so a description for the Tubercle Bacillus will be equally applicable to every variety of Bacterial Infection.

As a means whereby the inoculation of Bacterial Vaccines, and more especially Tuberculin (T.R.) can be adequately controlled, the opsonic index would seem to have met with its greatest utility as far as its application to practical medicine is concerned. Before its significance in this respect was appreciated, there can be little doubt that much unintentional harm was done by the, so to speak, haphazard inoculation of tuberculous patients with a Tubercle Vaccine.

The/

The application of the opsonic index in this connection can best be demonstrated by referring briefly to the effects produced thereon by the inoculation of a Bacterial Vaccine. Thus, for example where a single dose of a Bacterial Vaccine is inoculated, we have in the first instance a distinct fall in the opsonic index for that organism of which the vaccine consisted - the negative phase -. It is also found that the extent and duration of this negative phase varies within certain limits according to the dose of the vaccine; thus when the dose is small the negative phase is likewise slight, where, however, a large dose of vaccine is inoculated the negative phase is very marked, and may persist for a very considerable period, and coincident with this, constitutional symptoms, such as rise of temperature, increased pulse rate and respirations, may be developed. When only a single dose of Vaccine has been given the negative phase is succeeded by a rise in the opsonic index - the positive phase - to an extent above that recorded previous to inoculation. The time intervening between Inoculation and the establishment of this positive phase varies very considerably in different cases. In some it may be developed/

developed in 24 to 48 hours after inoculation whilst in others there may be an interval of 14 days. In a series of 160 cases of Phthisis investigated by Lawson and Stewart, 5% showed a persistent negative phase 14 days after inoculation. These observers also found that neither the temperature, pulse nor other clinical manifestations of the disease could be taken as in any way indicating the duration of the negative phase. Once established the positive phase tends to persist for some time gradually, however, subsiding to its original level. As a rule the duration of the positive phase resulting from a single inoculation of Vaccine is from 10 days to a month. If we now consider the effects on the opsonic Index resulting from a series of inoculations we find that a cumulative negative phase, i.e., a progressive fall in the opsonic index is very easily produced, whereas it is found a matter of the greatest difficulty, if not entirely impossible to effect a cumulative positive phase, at least with the Tubercle Bacillus. It is here that the regular estimation of the opsonic index finds its most useful application, for in this way and in this way only, can any definite and certain indication be obtained as to the exact time at which reinoculation can be safely/

safely resorted to. Thus, for example, if a second inoculation be given before the primary negative phase has given place to the positive phase, a still further lowering of the opsonic index results, a lowering which is manifestly detrimental to the patient; if, on the other hand, the positive phase is allowed to become well established and a second inoculation only resorted to when it is found that the opsonic index is again showing signs of declining, the negative phase necessarily induced as a result of this inoculation is found to be very slight indeed, and thus the benefit accruing from the positive phase subsequently developed is correspondingly great as this second positive phase is to a certain extent, so to speak, superimposed upon the previous one. It would seem, however, that in the case at least of the Tuberculo-opsonic index the limit of this superimposition of positive phases is soon reached so that it is impossible as has already been stated to produce a steady cumulation of positive phases. What can be attained however in the vast majority of cases, provided care be taken that the dose and interspacing of inoculations are appropriately guided by the regular estimation of the opsonic index, is a maintenance of the opsonic index at/

at a very considerably higher level than that obtained previous to inoculations. As time goes on it may be found necessary to increase the dose of Tuberculin (T.R.) but here again the only accurate indication is to be found in the regular estimation of the opsonic index. A great deal of evidence has already been recorded in this connection and on almost every hand the necessity of regularly estimating the opsonic index has been not only confirmed, but emphasized by the various observers. It will thus be seen that the opsonic index, as the only means whereby the inoculations of Bacterial Vaccines can be adequately controlled, has a very important practical application in Therapeutics.

We shall now discuss briefly the third and last point - namely -

III. IS VACCINE TREATMENT OF DISEASE JUSTIFIED BY THE RESULTS ALREADY RECORDED?

There are one or two considerations which make it a matter of some difficulty to give a definite/

definite answer to this question. Thus, for example, we find that, whereas, perhaps, the larger number of recorded results have been obtained in conjunction with regular estimations of the opsonic index, in others, however, no such regular estimations have been made. Then again, very considerable doubt would seem to exist regarding the exact dose of Tuberculin (T.R.) necessary to ensure the most satisfactory results; one is surprised on reading the large number of results which have already been recorded, to find so much variation in the dose of Tuberculin employed by different observers. It may be mentioned in passing, that Koch's New Tuberculin (T.R.) is standardized in relation to the weight of dried tubercular powder contained in a given volume of fluid: in what follows therefore, the weights given have reference to the tubercular powder only, and also except where otherwise stated, the term tuberculin has reference to Koch's New Tuberculin (T.R.)

Wright has laid it down as a general rule, that the dose of tuberculin should be the smallest capable of eliciting a satisfactory response on the part of the machinery of Immunisation. In Wright's experience $1/600$ mgm. is quite a sufficient dose for a primary inoculation, and in many cases it will be found that $1/1000$ mgm. will suffice. This view, as to dosage, is confirmed by many observers and especially/



especially by those who have practised inoculation in conjunction with careful and regular estimations of the opsonic index. If we now consider the dosage commonly employed by different observers, we find that $1/200$ mgm. and even $1/100$ mgm. are by no means uncommon and in a number of cases of Genito-urinary Tuberculosis recorded by Pardoe, the dose of tuberculin was commonly increased to $\frac{1}{4}$ or $\frac{1}{2}$ mgm. and in a few cases as much as 1 mgm. had been given. When such an extraordinary variation in the dosage exists, it is not to be wondered at if the results are of a somewhat varied character and yet, taken all over these would seem to be very much in favour of a more extensive use of Tuberculin in Tuberculous disease. There is one other point, however, which calls for special mention, and on which very considerable doubt would also seem to exist and that is the question of what cases are really suitable cases for Tuberculin treatment. In this connection definite rules have been laid down by Wright and as most, if not all of these have met with ample confirmation from other sources, it would perhaps be of some value to briefly refer to these here.

In the first place, in no case should inoculation be resorted to until several estimations of/

of the tuberculo-opsonic index have been made and it has been thus ascertained whether the latter is regular and steady or fluctuating from day to day. If of the former variety and low, inoculation can be safely commenced in conjunction with regular estimations of the opsonic index. As an initial dose $1/1000$ or $1/600$ mm. should be inoculated and a second inoculation should not be given until there is evidence that the positive phase induced by the initial dose is beginning to fall away. A reinoculation of the same dose is then resorted to and provided this be the proper dose, it is found that the negative phase is, in this case, less well marked than with the primary inoculation, and the positive phase as a result will be more rapidly produced. Should the contrary, however, be observed then one is justified in concluding that the dose has been too large, and this ought to be diminished in subsequent inoculations. In this way the correct dose is readily ascertained, and with correct interspacing of doses in subsequent inoculations, the tuberculo-opsonic index can be steadily maintained at a high level. When such a course is followed the very best results can be anticipated, and judging from those already recorded, there can be little doubt that in Tuberculin we/

we have a therapeutic agent of the very greatest value. The cases which naturally fall into this class comprise a very large proportion of tuberculous infections, including almost all varieties of localised infection, characterised by an absence of constitutional disturbance, also many early cases of Phthisis and almost all the more chronic varieties of this disease.

In the second variety of cases in which we have a regular index, but in which this is found to be normal or higher than normal the advisability of resorting to Tuberculin inoculation would seem to be more doubtful. Taking into account all the evidence, it would seem that in the first instance at all events, inoculation should not be resorted to, but rather that such a case should be watched carefully and the opsonic index regularly estimated. Should progress in the direction of recovery be made, then nothing further should be done, if on the other hand, the case should remain stationary, inoculation should be resorted to with the greatest care, only a very minute dose, $1/2000$ mcm. or even less being given in the first instance, and the effects of this very carefully studied, the question of continuing the inoculations depending entirely on the effects produced/

produced by the initial dose.

In the third variety of cases in which the opsonic index is fluctuating from day to day, and into this class also fall cases which are obviously acute with marked constitutional disturbance, even though they may present a persistently low index, the advisability of Tuberculin inoculations is entirely contra-indicated. In these cases the primary treatment should be directed on general lines, in an endeavour to arrest the fluctuation arising from the constant auto-inoculation from the seat of infection. Should these measures be successful and the opsonic index settle down and become regular, tuberculin inoculation may then be resorted to with the greatest care. In the other variety of cases in which the symptoms are obviously acute with marked constitutional disturbance, and where the opsonic index is persistently low, we are bound to conclude that the machinery of Immunisation is already taxed to the uttermost, and that, in all probability, inoculation would only result in the production of a still further lowering of the opsonic index. However, even here in so far as these cases are usually of the most hopeless variety and if left to themselves almost invariably terminate fatally, inoculation/

inoculation might be tried as a dernier ressort and the fact that a few cases are on record where a favourable result has been obtained by this means gives additional support to this view. The very greatest precaution must however be exercised, and only the most infinitesimal dose, about 1/6000 mgm. given in the first instance, the effects of which must be studied with the greatest care.

Such then are the chief considerations which one must have constantly before them in resorting to the treatment of Tuberculous Infections by the inoculation of Tuberculin. Where due attention has been given to these considerations, and the dose and interspacing of inoculations controlled by the regular estimation of the opsonic index, the results have been of a particularly encouraging nature, and even in cases where the dosage has been excessive or where adequate control has been wanting, the results are wonderfully uniform, and in many cases in the highest degree encouraging. The impression received from a perusal of the general facts already recorded from many sources indicates that in time, Tuberculin Therapy will prove, as Wright has stated a very valuable asset to medicine. Much will, however, require to be done ere it can be expected to meet/

meet with general acceptance, the laborious nature of the procedure at present involved limiting its application to a very great extent. With the lapse of time, however, and the steady accumulation of accurate observations, it may then become possible to formulate some definite rule as to the limits of dosage, and most satisfactory interspacing of inoculations, as a result of which, the regular estimation of the opsonic index will only be necessary, in the small minority of cases calling for such treatment.

Not the least interesting point raised by the introduction of Tuberculin Treatment, is the question of its utility as a prophylactic measure in cases of natural and hereditary predisposition to Tuberculosis, but meantime, the evidence accumulated in this connection is too limited, to permit of any definite statement being made. Should, however, adequate proof be forthcoming of its benefit in this connection, and the evidence so far recorded is certainly of an encouraging nature, the question thereby opened up is one of immense importance. If, for example, by a systematic examination of the tuberculo-opsonic index in all cases, where there is reason to suspect a predisposition to Tuberculosis, it were possible to overcome this predisposition by increasing/

increasing low indices by appropriate inoculations. The benefits thus conferred on humanity would be immeasurably great.

A few words with regard to the Vaccine treatment of other Bacterial Infections, and we are finished. Of these, Staphylococcal and more especially in this connection, Acne, Furunculosis and Syccosis, have been extensively treated by appropriate vaccines, and the results of such treatment have, in almost every case, proved of a highly satisfactory nature. Here also, in order that the most satisfactory results may be obtained, it is advisable that the Staphylococcal Opsonic Index should be estimated from time to time, but in this case, the importance of such examinations is not so great as the negative phase is of shorter duration and the positive phases show a much greater tendency to become superimposed, than in the case of the Tubercle Bacillus. The results of Typhoid inoculation introduced by Wright and since carried out so extensively in the army as a prophylactic measure, are too well known to call for any special mention here. Many other Bacterial Infections are now being treated on similar lines, and in many of these, the results are also encouraging, but so far, these recorded are too limited to make any/

any general statement of much value.

So much then for the general facts regarding opsonins, their nature, mode of action, and utility, so far as these are known at the present time. In the foregoing sketch an attempt has been made to accumulate from different sources all the general facts, and as far as possible to indicate, where diversity of opinion exists, what would seem to be the most likely conclusion suggested by a careful consideration of the general evidence relating to the point at issue. The sketch is necessarily brief and in parts, points of greater or lesser importance may have been inadvertently missed, but in extenuation it is claimed, that the literature appearing from time to time has naturally assumed considerable dimensions and is, in addition, so scattered, that it is now a matter of considerable difficulty to accumulate all the facts without making some well nigh unavoidable omissions. That much more will be heard of opsonin in the future, I have little doubt, but, at the same time, it must be admitted, that much more extended investigation and careful observation will be necessary, ere its real value can be properly appreciated. A short summary of the general conclusions arrived at in the foregoing sketch is appended.

SUMMARY OF THE GENERAL FACTS REGARDING OPSONINS.

1. Opsonins are normal constituents of the Blood.
2. Their presence would seem to be entirely limited to the Serum.
3. They are thermolabile.
4. They are relatively heliostable (evidence in this connection is, however, somewhat uncertain.)
5. There is reason to believe that they are specific separate opsonins existing for different varieties of Bacteria.
6. They probably belong to that class of elements known as Complements.
7. On the other hand, there is evidence that they do not belong to that class of elements known as amboceptors or immune bodies.
8. Their mode of action is that of combining with the Bacteria, thus preparing them for Phagocytosis.
9. In the process of Phagocytosis thus induced the leucocytes are considered an indifferent factor.
10. As to where these elements are produced, no certain/

certain knowledge is at present on record, but several authorities are of opinion that they are of leucocytic origin.

11. They are present in many secretions of the body but of these the most important, from the point of view of practical medicine, is the milk.
12. In conditions of disease resulting from a definite Bacterial infection, the normal opsonins are modified to a greater or lesser extent for that organism only, which is concerned in the production of the diseased condition: they may be increased or diminished.
13. Where a condition of immunity is established and also in some of the foregoing conditions of disease, the opsonic content of the blood becomes altered in character in most cases in the direction of a very considerable increased opsonic power.
14. How far this is due to an increase in the normal opsonins and how far to the production of a distinct and separate element, closely allied to normal opsonin is at present a matter of dispute.
15. Similarly whether this element is in reality a fresh/

fresh production or merely a modification of the normal opsonins, is also a matter of dispute.

16. That a new element is formed can be demonstrated by reason of the fact that it is definitely thermostable.
17. This new element has been called "The opsonic body found in immune serum" by most authorities. Leishman, however, believes the term "Stimulin" most applicable.
18. It is eminently specific only acting on that organism concerned in the production of the condition of Immunity.
19. There is evidence that it is largely heliobable.
20. Its action would also seem to be that of combining with the Bacteria, thus preparing them for Phagocytosis.
21. No certain knowledge is on record as to where in the animal body it may be produced.
22. The opsonic index for many different varieties of Bacteria varies from .80 to 1.20 in normal healthy individuals.
23. There is, however, relatively little variation from day to day.
24. That/

24. That it is affected to a slight extent by exercise and the ingestion of food would seem to have been fairly established.
25. The opsonic index in cases of Bacterial infection is of too variable a character to be of great value in the diagnosis of disease.
26. To various procedures which have been advocated to supplement the opsonic index in diagnosis, various objections attach.
27. In conjunction with the inoculation of a very small dose of Tuberculin, we have, however, a fairly delicate test for the presence of Tubercle.
28. Its greatest practical value is to be found in its application as a certain index by which the therapeutic inoculations of vaccine can be systematically controlled.
29. In so far as the results already obtained with vaccine treatment, are of a particularly encouraging nature, a wider application of such treatment would seem to be justified, provided due attention is accorded to the various considerations which have been stated in the text.
30. The question of Tuberculin inoculation as a prophylactic measure in all cases where a predisposition to Tuberculosis is suspected, has been suggested.

PART II.

In which a description is given in detail of the various experiments which have been personally carried out by the writer.

Before discussing the various experiments individually it is proposed in the first instance to give a short description of the technique involved by the different experimental procedures which it has been necessary to employ. Except where it is otherwise stated, the method employed in estimating the opsonic content of different sera, has been practically that of Wright, with a few modifications in some of the minor details which have suggested themselves to the writer or been suggested to him by other workers. In all cases the corpuscles employed were those of the writer. To obtain these a piece of stout glass tubing of about $\frac{1}{3}$ rd in. diameter and about $4\frac{1}{2}$ ins. long is taken and sealed at the one end in a Bunsen flame, thus making a convenient tube for purposes of centrifugalisation. A permanent mark is placed on this about $3\frac{1}{4}$ ins. from the sealed extremity and a similar mark about $\frac{3}{4}$ in. higher up, that is, about 4 ins. from the sealed extremity. This tube is then filled up to the $3\frac{1}{4}$ " mark with citrated saline solution, i.e. sod. citrate .80% in a .85% solution of sod. chloride. Blood is run direct from the finger into the citrated saline solution until the 4" mark is reached/

reached; the whole is then well shaken and centrifuged for about 10 minutes in an Electric centrifuge. The supernatant fluid is then pipetted off and the tube filled up with normal saline solution and the whole again well shaken and centrifuged for about the same time as previously. The supernatant fluid is then pipetted off as carefully and completely as possible. The upper layers of the deposit, familiarly known as the blood cream are then taken up in a long glass pipette and transferred to a suitable vessel, covered and laid aside till required. For collecting the serum an ordinary glass capsule is used. This is made from a piece of thin glass tubing about $\frac{1}{6}$ th in. diameter. Both extremities are drawn out in a Bunsen flame to form capillary ends, the finished capsule thus comprising a central portion conveniently about 1" long and of the same diameter as the original glass tubing, and at either extremity, a capillary portion of about the same length. In making these, the entire capsule is heated to a red heat and both extremities at once sealed and only broken immediately before using, thereby ensuring the perfect sterility of the capsule.

A/

A capsule of this description is filled about $\frac{2}{3}$ rds. full with blood and the ends sealed. It is allowed to stand for 10 or 15 minutes till clotting takes place and is then centrifuged to hasten and complete the separation of the serum. For this purpose the ordinary electric centrifuge was used, some cotton wool having been placed in the bottom of the centrifuge tube to form a convenient bed for the capillary end of the capsule. At first the "recurved" capsule described by Wright was employed and this was hung in the hand centrifuge for purposes of centrifugalisation. Experience however, showed, that with a capsule of this description breakages were not infrequent, and so it was abandoned in favour of the straight capsule, as this can be centrifuged quite as conveniently and with practically no risk of breakage provided the capillary portion is not too fine.

In the majority of the experimental work the *Staphylococcus Pyogenes Aureus* has been employed in estimating the effects of the different procedures on the opsonic content of the serum. In addition, however, observations have been made with *B. Coli.*, *B. Typhosis*, *Micrococcus Rheumaticus* and the/

the Tubercle Bacillus.

With the exception of the Tubercle Bacillus. Emulsions were prepared from a 24 hours culture growth of the organism, the latter being added to a 1.25% solution of sod. chloride and ground up in a small agate mortar. The resulting emulsion is then transferred to a convenient glass tube and centrifuged for a few minutes in order to bring down any Bacterial clumps which might not have been completely broken up in the mortar. To avoid, as far as possible, contamination of the contents, this tube was then covered by means of a glass capsule.

Fresh emulsions were made in the same way, about once a week, as it was found that the results obtained with a freshly made emulsion and one 2 or 3 days, or even a week old were exactly the same. All the emulsions were, however, shaken up daily and then centrifuged to ensure, as far as possible a homogeneous suspension of the Bacteria.

Emulsions of T.B. were prepared from the dried material obtained from cultures of the organism and which had been previously heated to 100° C. The method of preparation was exactly the same as in the case of the other organisms a 1.25% solution of sod./

sod. chloride being used. Fresh emulsions were prepared about every 10 days and daily shaking and centrifugalisation systematically carried out.

Having obtained the corpuscles, the serum, and the emulsion, a fine capillary pipette is next taken and a mark placed upon this, a convenient distance from the capillary end. Corpuscles, serum and emulsion, are then aspirated up to this mark, a small air bubble being taken up between each and in this way an equal volume of each of the three ingredients is obtained in the capillary portion of the pipette. These are then carefully ejected on to a clean glass slab by means of the rubber teat, reaspirated and ejected several times until thoroughly mixed, when they are finally ejected on to the slab. Having thoroughly mixed the three ingredients in this way, the mixture is transferred to a fine capillary tube which is at once sealed at both ends and placed in the incubator at a temperature of 37° C. for 15 minutes. Care was taken that each mixture was placed in the incubator at once, the exact time at which it should be removed being noted.

The/

The measuring pipettes were made from a piece of ordinary glass tubing about $\frac{1}{3}$ in. diameter, one end being drawn out into a fine capillary tube in a Bunsen flame. A separate pipette was kept for each variety of emulsion and these were thoroughly washed out with normal saline solution every time they were used and carefully dried before again using them.

At the expiry of the 15 minutes incubation, the capillary tube is removed from the Incubator, the ends cut and the contents blown out on to a clean slide and the film spread with a cigarette paper of convenient size. After drying at the room temperature the film is fixed over the Bunsen flame or by placing in the incubator for 15 minutes. The film is then stained with Jenner's or Leishman's stain, the former preferably, as it is quicker, and on the whole was found to give more uniform results.

In counting, never less than 30 leucocytes were counted. In practice 30 leucocytes taken in sequence were counted in the first instance and the number of ingested Bacteria for each individual leucocyte noted. Other 20 or 30 leucocytes were then/

then examined rapidly, without marking down the actual number of ingested Bacteria. In this way one could judge fairly accurately whether or not the average Bacteria per. leucocyte in the latter 20 tallied with the preceding 30. If there was any doubt on this point a second count of 20 leucocytes was made and the average taken for the 50 leucocytes.

In every case the control was furnished by the writer's blood, the degree of phagocytosis, i.e. the av. no. ingested Bacteria per leucocyte, obtained in this case being taken as unity and the result for the other blood calculated from this by dividing the degree of phagocytosis in this case, by that obtained for the control blood.

Estimations of the total number of leucocytes per c.mm. of blood were made in the ordinary way with the Thoma blood counting apparatus. For differential leucocyte counts, films were prepared in the ordinary way and stained with Jenner's stain. In making the counts 400 leucocytes were in each case counted, and the results are recorded as a percentage of the whole and in addition the total number/

number of each variety per. c.mm. of blood is given.

Except where otherwise stated the above procedures have been carried out in all the experiments in the manner already indicated and so it will be unnecessary to again refer to them in the case of individual experiments.

SERIES I.

The first series of experiments were carried out with a view to ascertaining the following:-

- A. Whether or not the number of one or other variety of leucocyte, per c.mm. of blood would correspond with the variations observed in the opsonic Indices of normal sera.
- B. Whether or not a serum showing a low index for one organism would show a correspondingly low index for all organisms.
- C. The variations shown by normal sera for different organisms.

A.

The variations in differential leucocyte counts in relation to variations in the opsonic Indices of normal sera.

For this purpose the Bloods of 12 apparently normal individuals have been examined. Total and differential leucocyte counts have been made in each case and the opsonic index estimated with *Staphylococcus P. Aur.* The writer's blood has, in each case, furnished the control, leucocyte counts being made in the same way. In making the differential leucocyte counts the following varieties have been recognised.

1. Polymorphonuclear leucocytes.
2. Lymphocytes.
3. Large mononuclear leucocytes.
4. Coarsely granular Eosinophyle leucs.
5. Mast cells.

In recording the results the lymphocytes and large mononuclears have been given separately but in addition have also been given combined, under the heading of Total Uninuclear cells.

The following are the results obtained in the case of the different bloods examined. The figures/

figures in red refer in each case, to the control blood. The total number of each variety of leucocyte per c.mm. of blood is alone given, as space would not permit of the percentage values being included in the same table. The average values for, on the one hand, the 12 different bloods and, on the other, the 12 separate estimations of the control blood are given in the bottom line of table.

Total/

Red figures = Control Blood

	Total Leucs. per C.mm. of Blood.	Poly- morph. Leucs. per C.mm.	Lym- pho- cytes. per C.mm.	L.Mo- nonu- clear leucs. per C.mm.	Total Uni- nucl. Cells. per C.mm.	Coarse Gran. Eosin- ophyl. Leucs. per C.mm.	Mast Cells. %	Stap- hylo- coc. opso- nic Index
1.	8000 6600	4592 4356	2208 1320	960 594	3168 1914	240 330	0 0	.99 1.00
2.	7000 8400	4032 4435	1834 2285	854 1176	2688 3461	280 504	0 0	1.17 1.00
3.	9000 7000	5895 4165	1755 1505	1080 980	2835 2485	180 280	1% 1%	1.16 1.00
4.	10200 8500	5712 4803	3213 1955	1071 1317	4284 3272	204 425	0 0	.90 1.00
5.	7500 7600	1875 4408	4312 1938	1218 608	5531 2546	75 646	.25% 0	1.00 1.00
6.	6500 7400	3396 3959	1966 2294	877 795	2843 3089	227 333	.5% .25%	1.10 1.00
7.	7500 6500	3975 3607	2512 1852	937 633	3449 2486	75 357	0 .75%	1.04 1.00
8.	7000 7600	4165 4750	2240 2166	490 342	2730 2508	105 342	0 0	.84 1.00
9.	7800 6800	4836 4182	2067 1700	741 714	2808 2414	78 204	1% 0	1.06 1.00
10.	7000 8800	3955 5104	1995 2684	840 748	2835 3432	210 220	0 .5%	1.20 1.00
11.	7600 7000	4484 3990	2318 1785	608 875	2926 2660	190 245	0 1.5%	1.07 1.00
12.	8800 7400	4444 4218	2772 1702	1144 1221	3916 2923	352 222	1% .5%	.97 1.00
AVERAGES.								
	7825 7460	4280 4330	2432 1932	893 833	3325 2765	185 342	.31% .37%	1.04 1.00

So far as these results go, it will be seen that neither the total number of leucocytes, nor any one variety shows a constant variation, corresponding to the variations in the Opsonic Indices. For purposes of comparison, each blood is compared with the control blood. It is, perhaps, unfortunate that the different bloods cannot be compared the one with the other, but, it will be evident that such a comparison would be entirely fallacious on account of the variations in the leucocyte counts of the control blood from day to day. In the present case, however, sufficient evidence is, I think, obtained by the comparison of each blood with its control to disprove any relationship between the Opsonic Index and the different varieties of leucocytes, at least, so far as these observations go. Thus, for example, in the case of Blood No.1. we find an opsonic index of .99 as compared with 1.00 for the control blood which Indices for all practical purposes may be taken as equal. On the other hand, the leucocyte counts show marked discrepancies in the number of every variety, except perhaps, in the case of the Polymorphs. In blood No.2. there is a corresponding difference in the leucocyte counts, except that in this case, the control blood shows the higher counts throughout, the opsonic indices/

indices, however, are not equal in this case, but show a difference of .17. Similarly with the other bloods, discrepancies existing to a greater or lesser extent in every case. Blood 5. is of particular interest on account of the very marked differences in the leucocyte count as compared with the Control Blood. This blood was obtained from a man apparently in excellent health, and the counts were verified by examination on other two occasions when almost identical results were obtained. The co-existence of an equal opsonic index with so marked discrepancies in every variety of leucocyte, would almost in itself seem to disprove any relationship between the two.

On one or two subsequent occasions where similar estimations have been made in connection with other experiments, these results have been confirmed.

B.

To ascertain whether a serum having a low Opsonic Index for one organism would have a correspondingly low Index for other organisms.

For/

For this purpose a number of Normal Sera have been examined, and in each case the Opsonic Index estimated for more than one variety of organism. The results of these observations are embodied in the following table:-

BLOOD.	STAPH.P.AUR.	T.B.	B.COLI.	B.TYPH.	M.RHEUM.
1.	.99	-	1.24	-	-
2.	1.16	-	1.02	-	-
3.	1.17	.70	.90	-	-
4.	1.07	.90	.91	-	-
5.	.90	1.16	1.03	1.17	-
6.	1.00	1.08	.90	.83	-
7.	1.10	1.10	1.04	.90	-
8.	1.04	1.00	.90	.90	.82
9.	1.20	1.00	.82	.70	-
10.	1.06	-	-	.94	1.00
11.	.97	-	.92	.92	-
12.	.84	-	-	.80	.94

These results would seem to indicate that a normal serum may show a high Index for one organism and yet show a low index for another organism.

Thus/

Thus in the case of No.1. the opsonic index for Staphylococcus is practically normal, whereas a very high index is recorded for B.Coli.

Serum 2. shows a comparatively high index with both organisms, that for Staphylococcus, however, being considerably higher than for B.Coli.

Serum 3. shows a high Index for Staphylococ. a comparatively low Index for B.Coli. and a very low Index for T.B.

Serum 4. shows a high Index for Staphylococ. a comparatively low Index for both T.B. and B.Coli.

Serum 5. on the other hand, shows a low Index for Staphcoc., but a high Index for the other three organisms.

Similarly with the remaining Sera variations in the value of the opsonic Indices for different organisms, being found in practically every case.

C.

The variations in the Opsonic Indices of a number of normal healthy individuals.

In this connection 32 Normal Bloods have been/

been examined for Staphylococcus P.Aur

10 for B.Coli.
 9 for T.B.
 8 for B.Typh.
 3 for Micrococcus Rheumaticus.

With the exception of 20 Staphylococcal and 2 T.B. Indices, these results have already been referred to in the previous experiment, and the individual indices will be found there in tabular form.

Taken together the following averages and variations were obtained:-

32. Staphcoc.	Av.	1.06	-	Variations from	.84	to	1.27
10. B.Coli	Av.	.96	-	"	"	.82	" 1.24
9. T.B.	Av.	.99	-	"	"	.70	" 1.16
8. B.Typh.	Av.	.89	-	"	"	.70	" 1.17
3. M.Rheum.	Av.	.92	-	"	"	.82	" 1.00

TOTALS.

62. Different organisms. Av. .96 - Variations from .70 to 1.27

In regard to the variations in the opsonic Index of one and the same blood from day to day, 5 estimations of the writer's opsonic Index for Staphcoc. were made. In every case the control was furnished by the blood of a normal guineapig, the same animal being used for this purpose on each occasion.

The following results were obtained:-

(1)	.99
(2)	1.02
(3)	.90
(4)	.90
(5)	.96

SERIES II./

SERIES II.

The following experiments were carried out to ascertain how far the opsonic content of normal serum would be reduced or increased, by keeping the serum for varying periods.

A. In contact with the corpuscles.

B. Separate from the corpuscles.

A. When it was desired to keep the serum in contact with the corpuscular elements, the serum was collected in the ordinary way in a sterile glass capsule, and immediately sealed. After clotting had taken place, the serum was centrifuged, and the capsule laid aside till required.

B. When it was desired to keep the serum separate from the corpuscular elements, the serum was collected and centrifuged as above. The capsule was then opened, and the clear separated serum transferred to another sterile glass capsule, which was immediately sealed and set aside till required.

In/

In every case the greatest care was taken to avoid any contamination of the sera with Bacteria. The finger from which the blood was collected was very carefully cleansed before puncturing, the prick-er was flamed before using, and the glass capsules in which the blood was collected rendered absolutely sterile by heating to a red heat prior to using. That these precautions were successful was evidenced by the fact that, in no case were any extraneous organisms found in the serum when the capsules were subsequently opened. In a corresponding set of experiments, to which reference will be made later, in which the sera were kept for varying periods in open capsules, there was abundant evidence of Bacterial contamination.

In the first series of experiments, the samples A and B, were collected at the same time, in the manner already described. They were then kept for the times specified, under exactly the same conditions, i.e., in sealed sterile glass capsules, at the room temperature, and exposed to the light. At the end of the specified time, the capsules were opened, and the sera tested in the ordinary way with fresh-washed leucocytes, and a suspension of Staphylococcus P.Aur, or B.Coli, and in one or two cases B. Typhoid/

Typhoid or M.Rheumaticus.

The phagocytic power of fresh serum estimated at the same time was taken as unity, and the Indices for the treated sera have been calculated from this in the ordinary way. A saline control was also done in every case, the results of which have not been included in the table, as in no case was more than a trace of phagocytosis observed, the indices varying from .02 to .10.

TABLE I./

TABLE I.

NO. of EX- PMT.	AGE of SERA	NATURE of BACTERIAL SUSPENSION	OPSONIC INDEX sample A. kept in contact with CORPUSCLES.	OPSONIC IN- DEX sample B.kept se- parate from CORPUSCLES.
1.	24 hrs.	Stphcoc.	1.16	-
2.	24 hrs.	B.Coli.	1.21	-
3.	8 days.	Stphcoc.	1.05	-
4.	5 days.	do.	1.02	.64
5.	do.	do.	.82	.40
6.	7 days.	do.	.89	.43
7.	do.	do.	1.05	.86
8.	do.	do.	1.21	.80
9.	do.	B.Coli.	1.19	.60
10.	do.	do.	1.03	.36
11.	do.	B.Typh.	1.03	.92
12.	14 days.	do.	1.04	.68
13.	do.	M.Rheum.	1.20	.50
14.	18 days.	Stphcoc.	1.15	.65
15.	do.	B.Coli.	.95	.40

Taken/

Taken all over, the results of these experiments are, I think wonderfully consistent. Thus of the 15 sera, kept in contact with the corpuscles only 3 show any loss of opsonic power. In seeking to explain these exceptions a question of some interest was suggested by the recollection that, in one or two cases some degree of haemolysis resulted from the keeping, but, unfortunately no note was made at the time as to which of the sera were thus affected. However, even including these exceptions, the average opsonic index for the 15 sera, is 1.06.

In the case of the 12 sera, kept separate from the corpuscles, the results are also, I think wonderfully consistent. Thus, in every case a decreased opsonic power is recorded, and in some cases, i.e., Nos. 5, 6, 10, and 15 this is very marked indeed. In Nos. 7, 8, and 11 the decrease is not so great. The average opsonic index for the 12 sera, is .60, which, it must be admitted is in very striking contrast to the average index of 1.06 obtained in the case of sera, kept for corresponding times in direct contact with the corpuscular elements.

In the next experiments, the sera were kept in an open glass capsule, exposed to the air for varying periods. In every case, the serum was kept in/
in/

in contact with the corpuscles. At the end of the time specified, there was abundant evidence of Bacterial contamination. The organisms usually found in the sera in these cases, were large thick Bacilli, which were readily phagocyted by the leucocytes. For this reason only the Staphylococcus was used in estimating the opsonic power, and in counting the Bacteria ingested, the extraneous Bacilli were not counted. The following results were obtained:-

TABLE II.

NO.	TIME KEPT.	OPSONIC INDEX for STAPHYLOCOCCUS.
1.	Kept 24 hrs.in open capsule	.91
2.	" 24 hrs. " " "	.80
3.	" 48 hrs. " " "	.60
4.	" 48 hrs. " " "	.62
5.	" 72 hrs. " " "	.82
6.	" 72 hrs. " " "	.86

These results are again in striking contrast to those obtained with sera likewise kept in contact with the corpuscles, but in a sealed capsule and/

and thus protected from Bacterial contamination. The average index for the 6 sera kept in open capsules, is .77 as compared with the average index of 1.06 obtained with the 15 sera, kept in sealed capsules.

The experiments embodied in Table 3 - vide infra - were carried out to ascertain what the result would be of incubating for 24 hours, with fresh-washed corpuscles, obtained in the ordinary way, a serum which had been kept separate from the corpuscular elements of the blood for a definite period, and which had thereby lost much of its opsonic power.

For this purpose two samples of Blood were collected and separated from the corpuscular elements in the manner already described. These were then set aside, and kept under exactly the same conditions for several days. On the day previous to that on which it was proposed to examine the sera, one of the capsules was opened, and about an equal volume of fresh-washed corpuscles added. The two were then thoroughly mixed, and transferred to another capsule which was immediately sealed and placed in the incubator at 37°C. for 24 hours. At the expiry of which time the capsule was removed from the incubator centrifuged, and the phagocytic power of the clear separated/

separated serum tested in the ordinary way. A control was furnished by the second sample of serum, which had not been treated with washed corpuscles. In each case the index was calculated from fresh untreated serum in the ordinary way.

TABLE III.

NO.	TIME KEPT.	OPSONIC INDEX for SERUM Kept separate from CORPUSCLES.	OPSONIC INDEX for the same SERUM, incubated 24 hrs. with fresh-washed CORPUSCLES.
1.	7 days	.86	1.30
2.	7 days	.80	.80
3.	7 days	.36	.44
4.	5 days	.64	.75
5.	5 days	.40	.31

With one exception these results indicate a rise in the opsonic power, as a result of incubation for 24 hours with fresh-washed corpuscles. Except in the case of No.1., the increase does not at first sight appear very great. If, however, it be taken into consideration that the addition of an equal/

equal volume of fresh-washed corpuscles suspended in normal saline solution must necessarily dilute the original serum to a considerable extent; this slight rise assumes a much more significant character, and in reality probably indicates quite a considerable increase in the total opsonic content. Unfortunately at the time, the significance of this dilution was overlooked, and proper precautions were not taken to accurately measure the quantity of washed corpuscles added, a fact, which might not only account for the relatively high index in No.1., but might also quite well account for the slight decrease in No.5.

Taking everything into consideration, I am inclined to think that, incubating with fresh-washed corpuscles a serum whose activity has been previously reduced by keeping, in all probability results in a considerably increased opsonic power.

SERIES/

SERIES III.

The next series of experiments were carried out to ascertain whether a serum inactivated with one variety of organism would be equally inactivated for all other varieties.

In this connection the following procedure was adopted:-

A considerable quantity of serum was obtained in the ordinary way and separated from the corpuscular elements. To this serum was added the organism with which it was proposed to inactivate it. In the case of the Tubercle Bacillus the dry pulverised tubercle growth was used for this purpose, but in the case of the Staphylococcus P. Aur., B. Coli, and B. Typh. the living organisms obtained from a 24 hours culture growth were employed.

The organisms were added to the serum and the two thoroughly mixed by grinding up in a small agate mortar until thorough emulsification was obtained.

In every case sufficient of the organism was added to form an emulsion of very considerable density.

The/

The resulting emulsion of serum and organism was then transferred to a glass capsule and placed in the incubator at 37° C. for $\frac{1}{2}$ an hour. On removal from the incubator it was centrifuged in an electric centrifuge for 1 hour, at the end of which time the Bacteria were found to have been completely separated. The clear supernatant serum was then pipetted off and tested in the ordinary way to ascertain what power it still retained of exciting phagocytosis with different organisms. This clear supernatant serum will be referred to as inactivated serum, the variety of organism concerned in the inactivation being stated. The deposit of Bacteria will be referred to as "opsonised".

In every case two controls were done

(a) with normal fresh untreated serum, the degree of phagocytosis thus obtained being taken as unity and the results obtained with the other treated sera calculated from this.

(b) A saline control, the results of which are not included in the tables as they were in every case negative, the indices varying from .01 to .06 as compared with 1.00 for normal serum.

The/

The results of a number of observations conducted on these lines are tabulated below.

TABLE I

NO.	NATURE OF SERUM	OPSONIC INDEX and ORGANISMS with which examined			
		Staph- coc. P.Aur	T.B.	B. Coli	B. Typh
1.	Normal Serum	1.00	1.00	1.00	1.00
2.	Serum inactivated with T.B.	.60	-	-	-
3.	" " " T.B.	.50	.08	-	-
4.	" " " T.B.	.62	.40	-	-
5.	" "Staphcoc.P.Aur.	.12	.35	-	-
6.	" " do	.23	.60	.50	.60
7.	" " do	.24	-	.22	.25
8.	" " B.Coli.	.57	-	.07	.03
9.	" " do	.72	-	.06	.60
10.	" " do	.46	-	.11	.25
11.	" " B.Typh.	.61	-	.35	.03
12.	" " do	.71	-	.40	.20
13.	" " do	.60	-	.20	.07

In/

In Table II - vide infra - these results have been subscribed more in detail. The order has also been changed in order to bring out more clearly the varying degrees of phagocytosis obtained with sera differently inactivated when subsequently tested with one and the same organism.

Table/

TABLE II.

NO.	NATURE OF SERUM	ORGANISM.	IN-DEX
1.	Normal untreated Serum	Staphcoc. P.A.	1.00
	Serum inactivated with Stpcoc. P. Aur.	+	.12
	" " " T.B.	"	.60
	" " " B. Coli.	"	.57
	" " " B. Typh.	"	.61
2.	Normal Serum	"	1.00
	Serum inactivated with Staph. Coc. P.A.	"	.23
	" " " T.B.	"	.50
	" " " B. Coli.	"	.72
	" " " B. Typh.	"	.71
3.	Normal Serum	"	1.00
	Serum inactivated with Staph. Coc. P.A.	"	.24
	" " " T.B.	"	.62
	" " " B. Coli.	"	.46
	" " " B. Typh.	"	.60
4.	Normal Serum	T.B.	1.00
	Serum inactivated with T.B.	"	.03
	" " " Staphcoc. P.A.	"	.35
5.	Normal Serum	"	1.00
	Serum inactivated with T.B.	"	.40
	" " " Staphcoc. P.A.	"	.60
6.	Normal Serum	B. Coli.	1.00
	Serum inactivated with B. Coli.	"	.07
	" " " Staphcoc. P.A.	"	.50
	" " " B. Typh.	"	.35
7.	Normal Serum	"	1.00
	Serum inactivated with B. Coli.	"	.06
	" " " Staphcoc. P.A.	"	.22
	" " " B. Typh.	"	.40
8.	Normal Serum	"	1.00
	Serum inactivated with B. Coli.	"	.11
	" " " B. Typh.	"	.20
9.	Normal Serum	B. Typh.	1.00
	Serum inactivated with B. Typh.	"	.03
	" " " Stpcoc. P.A.	"	.60
	" " " B. Coli.	"	.03
10.	Normal Serum	"	1.00
	Serum inactivated with B. Typh.	"	.20
	" " " Stpcoc. P.A.	"	.25
	" " " B. Coli	"	.60
11.	Normal Serum	"	1.00
	Serum inactivated with B. Typh.	"	.07
	" " " B. Coli.	"	.25

Table.

A study of the foregoing results suggests the following conclusions:-

That where a serum is inactivated by digestion with one variety of organism for $\frac{1}{2}$ an hour at 37° C. and these organisms subsequently removed by centrifugalisation,

1. The Serum is inactivated to a greater or lesser extent for all organisms.
2. The serum is, however, inactivated to a very much greater extent for that organism with which it has been previously digested; in some cases this inactivation is absolute.
3. If a number of sera are inactivated, each with a different variety of organism and subsequently tested with one and the same organism, that serum which has been inactivated with this organism will show a very diminished power of phagocytosis, whereas the other sera will still retain the power of exciting phagocytosis for this organism to a very considerable extent.

In a second series of experiments an attempt/

attempt was made to bring out these points on somewhat different lines and in these the following procedure was adopted.

Sera were collected in the ordinary way and inactivated with one variety of organism as in the preceding experiments. In subsequently testing the phagocytic power of these sera, however, a Bacterial Emulsion consisting of two distinct varieties of Bacteria was employed. Par example, in testing a serum which had been inactivated with Staph.coc.P.Aur. the bacterial emulsion comprised a mixture of Staph.coc.P.Aur and E.Coli or E.Typh. This modification was introduced to ascertain whether the resulting phagocytosis would be in any way selective for that organism with which the serum had not been previously digested. In using a double emulsion of this description one of the two varieties of organism in the mixture was always the same as that with which the serum tested had been inactivated.

A difficulty which at once presented itself arose from the fact that it was quite impossible to estimate numerically the relative proportions of the two varieties of organism, constituting this double/

double emulsion. This difficulty was, however, surmounted by doing in every case a control with normal untreated serum. In this way it was ascertained in what proportions the two organisms, constituting the double emulsion were phagocyted normally, and so in the case of a treated serum, by a simple comparison any alteration in these proportions was at once evident. By way of illustration, let us suppose that where a normal serum is tested with an emulsion, consisting of equal parts of a Staphylococcal Emulsion, and a B.Coli Emulsion, the average number of ingested Bacteria per leucocyte are Staphcoc. 6 - B.Coli 4, whereas on the other hand with a treated serum they are Staphcoc. 3 - B.Coli 4, then at a glance it is evident that the normal proportion is considerably disturbed. Taking each of the figures in the case of the normal serum as unity, and calculating the Index for the treated serum from this in the ordinary way, we have:-

Normal serum Indices - Staphcoc. 1.00 - B.Coli 1.00.

Treated " Indices - " .50 - B.Coli 1.00.

In counting the slides 30 consecutive leucocytes were counted, and the number of each variety of ingested organism noted.

In/

In the large majority, a number of each variety was found to have been ingested by the individual leucocyte. A saline control was also done in every case, but as these were all negative, the results are not included in the subjoined table.

By way of illustration one experiment is given in detail.

Expmt I.

EXPERIMENT I. ---

		AV.NO.INGESTED BACT.PER.LEUC.	INDEX
Washed Corpuscles	2 vols.		
Normal Serum	1 vol.	= Stpcoc. = 4.9 B.Coli = 3.7	= 1.00 1.00
Staphcoc.Emulsion	1 vol.		
B.Coli Emuls.	1 vol.		
Washed Corpuscles	2 vols.		
Serum inactivated		= Stpcoc. = 1.2 B.Coli 2.7	.24 .73
with Staphcoc.	1 vol.		
Staphcoc.Emuls.	1 vol.		
B.Coli Emuls.	1 vol.		
Washed Corpuscles	2 vols.		
Normal Saline Sol.	1 vol.	= Stpcoc. .03 B.Coli .30	.006 .08
Staphcoc.Emuls	1 vol.		
B.Coli Emuls.	1 vol.		

The/

The results of a number of experiments carried out in exactly the same manner, the same proportions of the various ingredients being used, are embodied in Table III.

To save complications no mention is made in the Table regarding the composition of the double emulsion. In every case, however, it comprised:-

- (a) 1 vol. of an emulsion of the same organism as that with which the serum had been inactivated, the index for which is given in red figures.
- (b) 1 vol. of an emulsion of some other variety of organism. In the case of a serum inactivated with Staphcoc. being tested, this other organism was always B.Coli., in the case of sera inactivated with B.Coli. or B.Typh. this other organism was always Staphcoc. The indices for these are given in black figures.

Table/

TABLE III.

No.	NATURE OF SERUM			INDICES FOR DBLE.EMULS.	
				Organ-ism (a)	Organ-ism (b)
1. Serum inactivated with Staphcoc.P.A.				.24	.73
2. " " " B.Coli				.24	.90
3. " " " B.Typh.				.26	.89
4. " " " Staphcoc.				.06	.28
5. " " " Staphcoc.				.30	.50
6. " " " B.Coli				.20	.60
7. " " " B.Typh.				.50	.70
8. " " " Staphcoc.				.32	.60
9. " " " B.Coli				.37	.67
10. " " " B.Typh.				.54	.63
11. " " " B.Coli				.54	.51
12. " " " B.Typh.				.37	.63
Average indices for the 12 inactivated Sera.				.33	.64

The results of these experiments confirm in a somewhat striking manner, those previously obtained with single Bacterial Emulsions. Thus, it will be seen, that even when an Emulsion consisting of/

of two distinct varieties of Bacteria is employed in testing the phagocytic power of a serum previously inactivated with one of these varieties, the resulting phagocytosis demonstrates clearly, that the opsonic power of a serum so treated, has been reduced to a much greater extent for that organism with which it was inactivated, than for the other organism with which it was not previously treated.

In a further series of experiments a few observations have been made with the deposits of "Opsonised" Bacteria, and these will be referred to briefly.

I. The serum having been pipetted off as completely as possible, the deposit was then thoroughly mixed up with Normal Saline Sol. sufficient of the latter being added to bring the mixture to its original bulk, i.e. a quantity corresponding to the serum pipetted off.

The mixture was then centrifuged for 1 hour and the supernatant clear saline tested in the ordinary way to ascertain if any opsonic power had been acquired, as a result of washing with the "Opsonised" Bacteria. The following are the results of a few experiments carried out in this way. The indices representing the phagocytic power of the saline so treated/ have

treated, have been calculated in the ordinary way from that of normal fresh serum.

EXPERIMENTS.

			Index
1. Saline in which opsonised		EMULS.	
Staphcoc. were washed.	+	Staphcoc.	= .37
" " "		B.Coli.	.22
2. Saline in which opsonised			
Staphcoc. were washed.	+	Staphcoc.	= .40
Saline in which opsonised			
T.B. were washed.	+	"	= .30
3. Saline in which opsonised			
Staphcoc. were washed.	+	T.B.	= .71
Saline in which opsonised			
T.B. were washed.	+	T.B.	= .29
4. In this case, the phagocytic power for normal fresh			
serum was not estimated, and so the results can			
only be given as the average number ingested			
Bacteria per leucocyte.			Av.No. ingested Bact.per leuc.
Saline in which opsonised			
Staphcoc. were washed.	+	Staphcoc.	= 11.1
Saline in which opsonised			
B.Coli. were washed.	+	"	= 4.4
Saline in which opsonised			
B.Typh. were washed.	+	"	= 5.4

In all the above experiments controls were also/

also done with ordinary normal Saline Solution in which no Bacteria had been washed, but the results in all of these were markedly negative, the indices ranging from .01 to .04.

These observations indicate that, when "Opsonised" Bacteria are added to normal Saline Sol., they impart to the latter a certain degree of opsonic power, and there is also evidence that the opsonic power thus imparted to the saline is not equal for all varieties of organisms, but would rather seem to be greater for that organism from which the saline has acquired its opsonic power. The results obtained in Experiment 3. however, do not bear out this latter statement.

5. The deposit of "Opsonised" Bacteria were mixed with normal Saline Solution and the mixture centrifuged as above. The supernatant saline was then pipetted off and the deposit again mixed with Saline Solution and ground up in a mortar till thoroughly emulsified, and the resulting Emulsion then tested with ordinary washed corpuscles without the addition of any serum. This emulsion is referred to as - Emulsion of "Opsonised" Staph-cocci or B.Coli. as the case may be. A control was done with an Emulsion of ordinary/

ordinary untreated Bacteria. The following are the results:-

- | | |
|-------------------------------|--------------------------|
| A. Washed Corpuscles. | Practically every leuco- |
| Emulsion of opsonised = | cyte gorged with cocci |
| Staph-cocci. | counting impossible. |
| B. Washed Corpuscles. | |
| Emulsion of opsonised = | " " |
| T.B. | |
| C. Washed Corpuscles. | |
| Emulsion of opsonised = | " " |
| B.Coli. | |
| D. Washed Corpuscles. | |
| Emulsion of opsonised = | " " |
| B.Typh. | |
| E. Washed Corpuscles. | Practically no phago- |
| <u>Ordinary Emulsion of</u> = | cytosis. |
| <u>Staphcoc.</u> | |

Also in the case of Washed Corpuscles and ordinary Emulsions of T.B., B.Coli., and B.Typh. practically no phagocytosis whatsoever was observed.

These results demonstrate very clearly that Bacteria digested with normal serum for half an hour and subsequently thoroughly washed in Saline Solution are very actively prepared for phagocytosis.

6. To/

6. To ascertain if such treatment impaired the vitality of the Bacteria to any marked extent, an ordinary agar culture tube was inoculated with Staphcoc.Pyog.Aur. which had been actively "opsonised" and washed in Saline, in the manner already described. At the end of 24 hours incubation at 37°C. a very copious and typical growth was found, and even in that time a very considerable degree of color had developed, more so than in the control tube of untreated Staphcoc.Pyog.Aur., of the same strain as the treated sample.

An emulsion in 1.25% Saline solution was made from the 24 hours growth of "opsonised" Staphcoc, and tested in the ordinary way with

- (a) Normal Serum.
- (b) Normal Serum inactivated by heating to 60°C. for $\frac{1}{2}$ an hour.
- (c) Normal saline solution.

A control was furnished in each case, by testing in the same way an emulsion of ordinary untreated Staphcoc., the results of these are given in black figures, those of the former i.e. obtained with the Emulsion of a 24 hours growth of opsonised Staphcoc. are given in red figures.

	INDICES.	
With Normal Serum	1.00	1.00
With Normal Serum inactivated by heating	.09	.04
With Normal Saline Solution	.02	.05

It will thus be seen that an Emulsion of Staph.Coc. prepared from a 24 hours growth of "opsonised" Staphcoc. gives practically the same results as the ordinary Emulsion of unprepared cocci.

SERIES IV.

Under this heading will be described a few experiments which have been carried out on animals, with the following objects in view.

- A. To ascertain what effects would be produced on the total and differential leucocyte counts and on the opsonic Indices of guineapigs, injected intra-peritoneally with Bone Marrow and Lymphatic Gland emulsions.
- B. To produce, if possible, in the guineapig a serum which would have active lytic properties towards the leucocytes of the rabbit.
- C. To develop in the guineapig a serum having definite immune properties towards *Staphylococcus Pyog. Aur.*

A.

The effects of injecting Bone Marrow and Lymphatic Gland Emulsions.

The/

The Emulsions were obtained from a freshly killed rabbit, the bone marrow from the Femur, the Gland Emulsion from the large mesenteric gland and spleen. In either case these were emulsified in warm normal saline solution and injected into the Peritoneal cavity of the guineapigs.

For this purpose three healthy full grown guineapigs were selected:-

GUINEAPIG	A	received injections of Bone Marrow Emulsion.
GUINEAPIG	B	" " " Gland Emulsion
GUINEAPIG	C	was untreated and served as control throughout.

In making differential leucocyte counts the following cells were recognised:-

1. Polymorphs.
2. Lymphocytes.
3. L. Mononuclears: included under this heading are the Vacuole cells comprising the larger proportion of the large mononuclear cells.
4. Coarsely granular Eosinophile cells.
5. Mast Cells: under this heading are noted cells with exceedingly coarse granules of a violet tint which in appearance were indistinguishable from the Mast cells of human Blood.

The following are the results of various observations made in this connection.

Before/

Before treatment was commenced the following Blood Estimations were obtained:-

Guinea pig	Total leucos p.cmm	Polv mph.	Lym cv tes	L.Mo nonu cr. %	Coarse Eosino philes	Masts	Ops. Ind. for Stpc.	Ops. Ind. for B. P.Aur. Coli.
JAN. 31. Before treatment.								
A.	10800	57%	26%	7%	9.5%	.5%	1.04	.83
B.	12600	55%	20%	8%	15.5%	.5%	1.07	.80
C.	13400	65%	22%	8%	4.5%	.25%	1.00	1.00
FEB. 1.								
Guineapig	A.	intraperitoneal inject.			Bone Marrow	Emuls		
"	B.	"			Gland	Emuls.		
"	C.	untreated.						
FEB. 4.								
A.	9800	54%	30%	6%	8%	1%	.90	1.5
B.	10000	52%	27%	4%	16%	.5%	1.09	1.4
C.	13600	62%	28%	6%	3%	.5%	1.00	1.00
FEB. 5.								
Guineapig	A.	intraperitoneal inject.			Bone Marrow	Emuls		
"	B.	"			Gland	Emuls.		
"	C.	untreated.						
FEB. 7.								
A.	10800	51%	22%	9%	16.5	1.5%	.96	1.10
B.	9000	47%	27%	10%	15%	1%	1.10	.90
C.	13600	56%	28%	10%	6%	.25%	1.00	1.00
FEB. 8.								
Guineapig	A.	intraperitoneal inject.			Bone Marrow	Emuls		
"	B.	"			Gland	Emuls.		
"	C.	untreated.						
FEB. 11.								
A.	12000	52%	25%	7%	15.5%	1.5%	.96	1.04
B.	9000	45%	37%	5.5%	12%	.5%	.88	1.08
C.	12800	57%	29%	10%	4%	.5%	1.00	1.00

If we compare the last of these estimations with the first we find that in the case of Guineapig A. treated with injections of Bone marrow emulsion there is a slight increase in the total leucocyte count and of the different varieties of leucocytes it is the coarsely granular Eosinophiles and Mast cells which are most markedly increased the percentage of Polymorphs being slightly diminished.

In the case of Guineapig B. treated with injections of Gland Emulsion there is a distinct fall in the total leucocyte count, apparently largely due to a marked diminution in the Polymorphs, whilst at the same time a very considerable increase in the percentage of Lymphocytes has resulted.

In the case of Guineapig C. which was untreated throughout neither the total nor differential leucocyte counts show any material alterations. If we now look at the opsonic indices which were estimated for two separate organisms throughout, we find that these have shown considerable fluctuations at different times and the values expressed in the final estimations are somewhat indefinite as on the one hand, the Index for B.Coli is very considerably increased whereas, on the other hand the Index for Staphylococcus is slightly diminished. Here again, therefore, as in the case of the observations made with human Bloods the results of which have already been/

been referred to, no definite relationship can be demonstrated as existing between the leucocyte counts, total or differential and the opsonic indices.

B.

To produce if possible in the Guinea-pig a serum having active lytic properties towards Rabbit's leucocytes. In this connection it has been shown by Metchnikoff, Besredka and others that a Leucolytic serum ~~can~~ be produced in a manner analogous to that employed in the production of Hæmolytic sera. In this case, however, suspensions of Leucocytes are injected instead of the red Blood Corpuscles. For this purpose emulsions of bone marrow or Gland Emulsion are usually injected in the manner in which these have been employed in the preceding Experiment.

I have made two separate attempts to produce such a serum in the guinea-pig but without success; one of the guinea-pigs developing peritonitis as a result of the injections died: in the other although 6 separate injections had been given, no lytic action could be demonstrated. For this reason and as time was limited, this experiment had to be abandoned.

It is a matter of regret that this endeavour was not attended with greater success as it was hoped/

hoped that some definite evidence might have been obtained regarding the possible leucocytic origin of opsonins by observing whether the phagocytic power of the serum was materially increased by the disintegration of the leucocytes resulting from the action of a leucolytic serum.

C.

To develop in the guineapig a serum having definite immune properties towards *Staphylococcus Pyog.Aur.* For the purpose of immunisation, two healthy guineapigs were selected. About $\frac{1}{2}$ to 1 C.C. of a *Staphylococcal* Emulsion which had been previously heated to 100° C. for 1 hour was injected intraperitoneally at intervals of 3 days. In all 8 injections were given. The opsonic Indices were estimated for *Staphcoc.* and *B.Coli*, normal human Serum serving as the control from which the other indices were calculated. The opsonic power in the case of immune sera was also estimated after heating to 60° C. and the index for this also calculated from the phagocytic/

phagocytic power obtained for normal untreated serum.

Throughout the Index for B.Coli was unaffected by the injections; in the case of the Indices for Staphylococcus, however, the following changes were observed.

Before injections were commenced the indices were.

Guineapig D.	1.25
" E.	1.21

The indices for Staphcoc. after the various injections were as follows:-

After 1st injection

Guineapig D.	.80
" E.	.76

After 3rd injection

Guineapig D.	1.12
" E.	1.16

After 5th injection

D. unheated	= 1.38	heated	= .19
E. "	= 1.65	"	= .31

After 6th injection

D. unheated	= 1.40	heated	= .32
E. "	= 1.50	"	= .33

After 8th injection

D. unheated	= 1.36	heated	= .25
E. "	= .70	"	= .30

After the 7th injection, guineapig E. developed a considerable degree of Ascites which in all probability/

probability accounts for the low index recorded after the 8th injection. No further injections were given but on two subsequent occasions, the following indices were recorded for Guineapig E.

6 days after the 8th injection

E. unheated = 1.50 heated = .22

12 days after the 8th injection

E. unheated = .64 heated = .40

Guineapig E still shows considerable Ascites.

It will thus be seen that in the case of Guineapig D, although some increase in the opsonic index is recorded with the unheated serum, on the other hand, the phagocytic power of heated serum depending on the presence of the true immune substance is not very great.

In the case of Guineapig E, the index has apparently assumed a fluctuating character analogous to what is found in cases of Acute Tuberculosis. The proportion of true immune substance would, however, seem to be greater in this case than in the case of Guineapig D.

Taking everything into consideration, I did not feel justified in regarding either of these sera as really good examples of Immune Serum, and so the observations/

observations which I intended carrying out, had the process of immunisation resulted in the production of a larger proportion of true immune substance, were abandoned. In themselves, however, these results are of some little interest, and as I shall have occasion to refer to one or two of them later, they have been included amongst the other experiments.

PART III.

In which a description is given of a simple and rapid method of estimating the Opsonic Power of the blood in health and disease.

PART III.

It is proposed to devote this section to a description of a simple and rapid method of estimating the Phagocytic activity of different Bloods in health and disease. In what follows, this method will be referred to as the "Modified Method" or M.M. to distinguish it from the ordinary Method or O.M.

The Modified Method is in reality a modification of Leishman's original technique. In a few words, it consists in mixing together a definite quantity of Blood, Citrate Solution and Bacterial Emulsion, incubating the mixture for a definite time in a Capillary tube, subsequently spreading and staining the film in the ordinary way. By this procedure a mixture of the Cellular elements of the blood, plus a definite quantity of Bacteria suspended in a diluted blood plasma, is obtained. The addition of the Sodium Citrate solution prevents clotting entirely, and even in the small quantity of blood used there are far more than sufficient Leucocytes present for purposes of enumeration. The method is quick/

quick and at the same time simple, and much time and labour is saved, since it is quite unnecessary to collect and wash the corpuscles separately. In practice, all that is required in the form of apparatus, is a Capillary pipette, and this is readily obtained by drawing out a piece of thin glass tubing in the bunsen flame. The capillary portion should be at least 6 ins. long, and for the purpose of measuring, a mark is placed on this about $\frac{1}{2}$ in. from the free end. Instead of the rubber teat, a piece of rubber tubing with mouthpiece is attached to the wide end, and by this means the requisite amounts of blood etc., are drawn up into the capillary portion of the pipette. This plan was adopted in preference to the rubber teat, as in measuring, the quantities could be more easily controlled in this way than by means of the teat. Blood is obtained from the finger or ear in the ordinary way. Two volumes are drawn up into the capillary portion of the pipette, the mark thereon serving to measure the volumes, and this is immediately followed by 2 volumes of a 1.5% solution of Sod. Citrate, and 1 volume of a suspension of the Bacteria in 1.25% Sod.-Chloride solution, i.e., the ordinary Bacterial emulsion. These ingredients are measured in the ordinary way, a small air bubble being/

being introduced between each of the volumes. The whole is immediately blown out on a clean glass slab and thoroughly mixed by reaspirating and blowing out several times. It is finally drawn up into the capillary portion of the pipette, the end of which is sealed in the flame, and the whole placed in the incubator at 37° C. for 15 minutes; at the expiry of which time the sealed end is cut, and the contents blown out on a clean glass slide, mixed, and spread in the ordinary way. The method of spreading with a cigarette paper already described, has subsequently been abandoned, and instead a thin cover glass substituted for this purpose. The film is fixed with heat and stained with Jenner's stain or Zeil Neilson in the case of the Tubercle Bacillus.

As in the ordinary method, a control is done in every case in exactly the same way with a normal blood, the degree of phagocytosis thus obtained being taken as unity, and the index for the other blood under consideration calculated therefrom. Such is the procedure adopted in every case where it is possible to obtain the blood direct from the patient, and incubate it immediately.

Where this is impossible, a slight modification is necessary. In this case the blood is drawn and/

and immediately mixed with Citrate Solution alone, i.e., without the addition of the Bacterial Emulsion. It is then drawn up into the capillary pipette, and the end of this sealed. This mixture will not clot, and can therefore be kept for as long as desired. It is desirable, however, that the final mixing with the Bacterial Emulsion should not be delayed longer than 2 or 3 hours. When it is convenient to do so, the sealed end of the pipette is cut and the contents blown out on a glass slab, and again thoroughly mixed. A fresh pipette is taken, and 4 volumes of the mixture of Blood and Citrate Solution measured off in the ordinary way, and 1 volume of the Bacterial Emulsion added; and the whole having been again thoroughly mixed, is finally drawn up into the capillary portion of the pipette which is sealed, and placed in the incubator at 37°C . for 15 minutes. When this procedure is adopted, the control blood should be collected at the same time, and in the same way as that of the patient. The two are then marked and kept under the same conditions until it is convenient to mix with the Bacterial Emulsion immediately prior to incubating. This precaution is necessary as there is evidence - vide infra - that the keeping results in a somewhat diminished phagocytic power. If, however./

however, the two bloods are kept for the same time and under the same conditions, the diminution is equal in each case, and the final result in no way altered.

A number of observations have been made with Blood and Citrate mixtures kept for varying periods from a $\frac{1}{2}$ hour to 3 hours, to which reference will be made later - vide infra.

Other observations have also been carried out to ascertain:-

- I. Whether or not the addition of a 1.5% Sod.Citrate Solution to the serum tested in the ordinary way with washed leucocytes would materially affect the result.
- II. A comparison of the results obtained with the ordinary method and with the Modified Method for the serum and Blood of the same individuals.
- III. The effect on the resulting phagocytosis of keeping the Blood Citrate mixture for varying periods.
- IV. In addition a considerable number of bloods obtained/

obtained from apparently normal healthy individuals have been examined by the Modified Method alone, to ascertain the normal range of variation in the Opsonic Index when estimated by this means.

- V. Also the Bloods of a number of patients suffering from a known Infective disease have been examined by the Modified Method alone, to ascertain whether the variations in the Opsonic indices in these cases observed with the ordinary method would be similarly observed with the Modified Method.

I/

I.

The EFFECT of adding SOD. CITRATE SOL. to SERUM when tested by the Ordinary Method.

For this purpose two sera were compared.

(a) In the pure state.

(b) Diluted with 1 volume Sol.Citrate Sol.

1.5%

The following are the results:-

		Av.No.Ingest- ed cocci per leuc.	Index.
Washed corpuscles	1 vol.		
Serum (control)	1 vol.	= 5.8	1.00
Stpccoc. Emuls.	1 vol.		
Washed Corpuscles	1 vol.		
Serum (control)	1 vol.	= 5.9	1.00
Citrate Sol. 1.5%	1 vol.		
Stpccoc. Emuls.	1 vol.		
Washed Corpuscles	1 vol.		
Serum A	1 vol.	= 6.7	1.15
Staphcoc.Emuls.	1 vol.		
Washed Corpuscles	1 vol.		
Serum A.		= 6.6	1.12
Citrate Sol. 1.5%	1 vol.		
Staphcoc. Emuls.	1 vol.		

In the above experiment two controls were necessary in order to eliminate any difference resulting from the dilution. Thus, the index for Serum A in the untreated state is calculated from the control Serum/

Serum also untreated, the index for Serum A treated with Sod. Citrate is calculated from the control Serum similarly treated.

For all practical purposes, the two indices obtained for serum A, on the one hand untreated = 1.15 and on the other hand, treated with Sod. Citrate = 1.12, may be taken as equal.

In the next experiment exactly the same procedure was adopted except that 2 vols. of Sod. Citrate Sol. 1.5% were added instead of 1 vol. as in the preceding experiment.

RESULTS.	AV.NO.INGESTED COCCI PER LEUC.	INDEX.
Control Serum untreated.	= 4.7	1.00
Control Serum + Sod.Cit.2 vols.	= 3.8	1.00
Serum B. untreated	= 5.3	1.13
Serum B. + Sod.Cit.2 vols.	= 4.2	1.10

Here again, the difference in the two Indices for Serum B. is so slight that for all practical purposes these may be taken as equal.

Both in the case of the Control Serum and Serum B. the av.no.ingested cocci per leucocyte is less/

less with the treated sample than with the untreated, a difference which is readily accounted for by the degree of dilution resulting from the addition of 2 Vols. of the citrate solution.

These experiments have been repeatedly confirmed with practically identical results.

I am, therefore, led to the conclusion that citrated serum acts in exactly the same way as ordinary untreated serum, due allowance being made for the dilution.

II/

II.

A comparison of the Indices obtained for Normal Serum by the ordinary Method, and for the whole Blood by the Modified Method.

For this purpose, the Opsonic Indices for Staphcoc. Aur. or T.B. (in two cases) of 12 apparently normal individuals were estimated on the one hand by the ordinary Method, and on the other, by the modified Method. The results are appended in Table I.

TABLE I.

NO.	SERUM.	ORGANISM.	INDEX O.M.	INDEX MOD.M.
1.	Serum A.	Staphcoc. Pvog. Aur.	1.00	1.01
2.	" B.	"	1.27	1.15
3.	" C.	"	1.14	1.09
4.	" D.	"	1.15	1.13
5.	" E.	"	1.13	1.18
6.	" F.	"	1.03	1.05
7.	" G.	"	1.01	1.03
8.	" H.	"	1.08	.45
9.	" I.	"	1.06	1.02
10.	" J.	"	1.00	1.04
11.	" K.	Tubercle Bacillus.	.97	1.14
12.	" L.	"	1.04	1.00

From a comparison of these Indices, it will be seen that with the exception of No.8. and to a lesser extent No.11. the results obtained with the modified method are practically the same as those obtained with the ordinary method. In No.8. the difference is so marked, that some technical error must have been introduced of sufficient magnitude to entirely vitiate the result.

III./

III.

The effects of keeping Blood citrate mixture for varying periods.

For this purpose one sample of blood was collected, mixed with citrate solution and Bacterial Emulsion and immediately incubated. Several other samples of blood were also collected and mixed with Citrate Solution alone. These were kept for varying periods and subsequently tested with a suspension of Staphylococ, in the manner already described. In the case of the control blood, a similar procedure was adopted, the different samples being kept for exactly the same time and under the same conditions. The indices for the blood tested are in each case calculated from the control mixture kept for a corresponding period.

The following observations have been made on these lines:-

1./

		Av.no.ingest. Index cocci per leuc.
1. Blood		
(control)	collected, mixed and incubated immediately.	7.3 = 1.00
do	Blood citrate mixture kept 1 hr. Bact. then added & incubated.	4.2 = 1.00
Blood A	collected mixed and incubated immediately.	8.3 = 1.13
do	Blood citrate mixture kept 1 hr. Bact. then added & incubated.	4.7 = 1.12

From the average number of ingested cocci per leucocyte, it is seen that keeping the blood citrate mixture for 1 hour, results in a somewhat diminished phagocytic power. This diminution is, however, so proportionate in the case of both the control blood and Blood A. that the resulting Index for Blood A., calculated from the control Blood kept for a corresponding time is practically unaffected. A considerable number of observations have been made in the same way with identical results

The following are a few examples:-

		Index.
Blood B.	immed. incubated	1.18
do	incubated after 1 hr.	1.17
Blood C.	immed. incubated	1.05
do	incubated after 3 hrs.	1.08
Blood D.	immed. incubated	1.03
	incubated after 3 hrs.	1.03

These/

These results demonstrate that the Index of a blood when the citrate and blood mixture has been kept for varying periods, is practically identical with the Index obtained when all the ingredients are mixed at the time of collecting and immediately incubated. It is necessary, however, that the Control Blood, from which this Index is calculated, should be subjected to exactly the same treatment, and kept for a corresponding time.

Regarding the extent to which the phagocytic power is reduced a few observations have been made with one and the same blood kept for varying periods. Thus:-

			Av. ingest cocci per leuc.
A. Sample	1	Collect. & immed. incub.	7.3
"	2	Blood Cit. Mixt. kept $\frac{1}{2}$ hr.	4.6
"	3	" " " " 1 hr.	4.2

In the next observation the Emuls. of Staphcoc. was much thinner, and in consequence the Av. No. Ingested Cocci per leuc. was considerably less; and here the difference observed in the case of samples kept for different periods is not so marked

Thus/

Thus:-

						Av. ingest cocci per leuc.
B. Sample	1	Collect.	&immed.	incub.		2.3
"	2	Blood	Cit.	Mixt.	kept $\frac{1}{2}$ hr.	2.2
"	3	"	"	"	" $\frac{1}{2}$ hr.	2.1
"	4	"	"	"	" 1 hr.	2.0
"	5	"	"	"	" $1\frac{1}{2}$ hrs.	2.1
"	6	"	"	"	" 2 hrs.	2.0

As reflecting in some measure on the technical accuracy of the modified method, the following two observations are given.

1. Blood A. collected and immediately incubated for 10 mins.

Av.no. ingested cocci per leuc.= 10.1

The same blood collected at the same time and immediately incubated for 20 mins.

Av.no. ingested cocci per leuc.= 23.2

		Cocci per leuc.
2. Blood A. + <u>2 Vols.</u> of Cit.Sol. + Emulsion		2.3
Same		
Blood + <u>1 Vol.</u> of Cit.Sol. + do		4.2

In the first of these observations the effect of incubating for double the time, on the resulting phagocytosis is brought out in a striking manner.

In the second the effect of diluting the same quantity of Blood. on the one hand with 2 vols. of/

of Citrate Sol., and on the other with only 1 vol., is shown in a striking manner by the resulting phagocytosis.

The following tables embody the results of a number of estimations of the Opsonic Indices of normal healthy individuals for different organisms.

The Modified Method was employed in every case.

Table/

TABLE I. contains the results of 27 Indices for
Staphcoc.Pyog.Aur.

TABLE I.

NO.	ORGANISM	INDEX	NO.	ORGANISM	INDEX
1	Staphcoc.P.A.	1.01	15	Staphcoc.P.A.	1.11
2	"	1.15	16	"	.92
3	"	1.25	17	"	.74
4	"	1.09	18	"	.90
5	"	1.13	19	"	1.00
6	"	1.12	20	"	.90
7	"	1.18	21	"	1.11
8	"	1.17	22	"	1.03
9	"	1.05	23	"	1.12
10	"	1.03	24	"	1.21
11	"	1.08	25	"	1.14
12	"	1.03	26	"	1.06
13	"	1.09	27	"	1.00
14	"	1.04	-		-

The/

The average index for the 27 bloods is 1.06, the variations being from .74 to 1.25.

On reference to the table it will be seen that only 1 blood shows an Index below .90 namely No.17 in which an index of .74 is recorded. It is thus evident that these results compare very favourably with those obtained by the ordinary method and to which reference has been made in another place, the results of which were - For 32 Bloods Average, 1.06. Variations .84 to 1.27.

TABLE II comprises the results obtained in the case of the Tuberculo-opsonic indices of 12 normal individuals.

TABLE II.

NO.	ORGANISM	INDEX	NO.	ORGANISM	INDEX
1	T.B.	1.14	7	T.B.	1.01
2	"	1.00	8	"	1.14
3	"	.99	9	"	.99
4	"	1.00	10	"	1.05
5	"	1.01	11	"	1.05
6	"	1.06	12	"	1.08

Average/

Average for the 12 Bloods is 1.04 Variations .99 1.14

Results previously obtained with the ordinary method.

Average for 9 Bloods .99 variations .70 to 1.16

The consistent nature and limited range of variation in the case of the 12 Indices estimated with the modified method is very apparent.

TABLE III. comprises the results of 8 normal Indices for B.Coli. estimated by the Modified Method.

TABLE III.

NO.	ORGANISM	INDEX
1	B.Coli.	1.06
2	"	1.02
3	"	1.06
4	"	.92
5	"	.92
6	"	1.05
7	"	.97
8	"	.97

Average/

Average Index for 8 Bloods '99 variations '92
1'06.

Results previously obtained with the ordinary method.

Average for 10 Bloods '96. Variations '82
to 1'24.

Here again the consistent nature and limited range of variation in the case of the 8 Indices estimated by the modified method is very apparent and contrasts very favourably with those previously obtained with the ordinary method.

Adding all these results together we have 47 normal indices for different bloods.

Average index of 47 Bloods 1'04. Variations
'74 to 1'25.

It is of particular interest that of the 47 Bloods examined only 1 index was obtained below '90.

Results previously obtained with the ordinary method.

Average for 62 Bloods '96. Variations '70
1'27.

In this case quite a large proportion of the individual Indices were below '90.

Taken/

Taken all over these results indicate that where the modified method is used in estimating the opsonic Indices of normal individuals the results thus obtained are quite as accurate as those obtained where the ordinary procedure is adopted.

V./

V.

Under this heading the Opsonic Indices of a number of patients suffering from a known infective disturbance have been estimated by the modified method. With one exception, all of the 30 cases examined were suffering from some variety of Tuberculosis. In the case of the exception, no definite diagnosis had been arrived at; reference will, however, be made to this later. The 29 cases of undoubted Tuberculosis comprised all varieties of the disease, Acute and Chronic, Pulmonary, Abdominal, Genito-urinary and Spinal lesions, Joint affections and one case of Middle Ear disease. At the time of examination, all the patients were under treatment, either in the wards of the Royal Infirmary or at the Convalescent Home.

In every case, the mixture of Blood, Citrate Solution and Emulsion of T.B. was made at the bedside and immediately incubated. As the use of an Incubator in one of the wards was kindly granted, the time elapsing between the mixing and placing of pipette in the incubator was never more than 2 or 3 minutes, and care was taken to allow the same interval to/

to elapse with the Control blood, which in every case was that of the writer. . . A detailed list of the various cases examined, the nature of the Tubercular lesion and the tuberculo-opsonic index will be found in Table IV. The most acute cases in which constitutional disturbance was marked, are given in red.

TABLE/

TABLE IV.

NO.	NAME	NATURE of TUBERCULAR LESIONS	T.B. OPSONIC IND.
1.	Hunter	Advanced Phthisis improving under treatment.	.53
2.	Miller	Advanced Phthisis acute active.	.54
3.	Bruce	Advanced Phthisis acute active.	.66
4.	Tiffney	Double apical Phthisis acute.	.44
5.	Scott	Chronic Tubercular ulceration of Bowels with Chronic Peritonitis, convalescent but still slight temperature.	.71
6.	Beveridge	Chronic Phthisis & subacute Pleurisy with effusion. Improved.	.67
7.	Campbell	Tubercular glands in neck, abscess over sterno-clavicular joint, acute pyrexial attack - pneumonia?	.58
8.	Wallace	Phthisis and Empyema.	.61
9.	McGurtrie	Advanced acute phthisis.	.56
10.	Bell	Advanced acute phthisis.	.45
11.	Duncan	Phthisis, 8 weeks treatment, greatly improved, still has slight temperature.	.74
12.	Wallace	Genito-urinary tuberculosis, tubercular testicle excised.	.55
13.	Anderson	Chronic Pulmonary & Abdominal Tuberculosis - convalescent.	.59
14.	Sutherland	Advanced acute Phthisis.	.56
15.	Campbell/		

TABLE IV. (contd.)

NO.	NAME	NATURE of TUBERCULAR LESIONS	T.B. OPSONIC IND.
15.	Campbell	Early apical Phthisis.	.59
16.	Lawson	Early apical Phthisis.	.61
17.	Aitchison	Early apical Phthisis.	.72
18.	Sutherland	Tubercular wrist, 4 months duration, passive treatment.	.79
19.	M ^C Leod	Chronic Tubercular Middle Ear disease, operated on.	.75
20.	Fawcett	Tubercular abscesses in axilla and neck.	.73
21.	M ^C Ateer	Potts disease.	.66
22.	Newman	" "	.73
23.	Blades	Tubercular dactylitis of toe, operated on, unhealthy wound.	.86
24.	Drysdale	Old Chronic fibroid Phthisis.	.78
25.	Wishart	Genito-urinary Tuberculosis.	.73
26.	Wishart	Tubercular hip joint disease discharging.	.72
27.	Thomson	Tubercular kidney also leg amputated for tubercle 8 years ago.	.72
28.	Carr	Old standing Phthisis.	.69
29.	Smail	Intra-Cranial Tumour, Tubercle?	.78
30.	Kelly	Old standing Tubercle Empyema, discharging.	.82

A study of these results reveals in a striking manner, the consistently low tuberculo-opsonic index obtained for almost every variety of Tubercular affection. These results are fully discussed in another place (*vide infra*) and it is therefore not proposed to make any further reference to them here.

PART IV.

Which is devoted to a discussion of PARTS II. & III.
and in which a theory is elaborated as a possible
explanation of the process of phagocytosis.

PART IV.

It is here proposed to discuss in detail the various experiments embodied in PARTS II. & III.

In the first series, estimations of the Opsonic Indices of 12, apparently healthy normal individuals, for *Staphcoc.Pyog.Aur.* in conjunction with total and differential leucocyte counts were undertaken, in the hope that some constant parallelism might be demonstrated as existing between the normal variations in the Opsonic Indices and the number of one or other variety of leucocyte. The fact that these observations are negative and that a number of equally negative results in the case of analogous observations have been recorded by others, would seem to indicate that no such parallelism exists. Still, I do not think that the lack of proof in this direction necessarily excludes the possibility of opsonins being in reality leucocytic products as in observations of this kind many variable factors are unavoidably introduced, capable of very materially influencing the results. Thus, for instance, where so many/

many different procedures are involved, the range of experimental error must necessarily be of some magnitude, and in addition, it is quite possible that differences in the secretory activity, if such in reality exists, of the cells of different individuals might account, to some extent, for variations in the results. On the other hand, a result, such as that obtained for Blood No.5., already referred to, is very suggestive as in this case, the opsonic index is apparently unaffected, although the percentage of polymorph and uninuclear leucocytes is practically reversed. Taking everything into consideration it is difficult to come to any definite conclusion, but I cannot help thinking that observations on these lines are so complicated by the introduction of so many variable factors, that one is hardly justified in attaching much weight to the results obtained in this way.

By the second series of Experiments proof is afforded that one Serum may show a comparatively low opsonic index for one organism and at the same time a comparatively high index for another organism. There are, I think, three possible explanations of this occurrence:-

1. Experimental Error.
2. A difference in the virulence of the various organisms.
3. That the opsonins of normal serum are specific.

The first of these, namely that of experimental error, cannot be entirely eliminated, but the fact that in some of the sera tested an index as low as .70 was obtained for one organism, and at the same time an index as high as 1.20 for another organism, would seem to suggest that some other factor must be at work. The range of experimental error in estimating the opsonic index is undoubtedly considerable, but certainly not of sufficient magnitude to cause a difference of .50 in the case of two separate estimations. Some of the minor differences might possibly meet with a sufficient explanation on the grounds of experimental error, but I am convinced that this factor in itself, does not adequately explain so marked a difference as that referred to above.

The second of these suggested explanations can also, I think, be disposed of in so far as a difference in the virulence of the organisms would necessarily effect different bloods to the same extent. That is to say, where several bloods are compared with more than one variety of organism, and the same Bacterial Emulsions used in each case, one would expect all the bloods to show a proportionately low index for that organism with the greater virulence. A reference, however, to the results obtained/

obtained in this connection shows clearly that no such definite behaviour exists, but that the high or low indices occur quite indiscriminately with one variety of organism or another. The density of the different emulsions used is another factor relative to the Bacteria which might possibly influence the results, as this varied in each case from day to day. For want of adequate control this influence cannot, in the present case, be definitely excluded, but I hardly think it would suffice to entirely explain the differences in every case. This brings us to the third suggestion, namely, the question of the specificity of the opsonins of normal serum. This question will be fully discussed in another place, and so it will only be necessary to state here, that there is sufficient evidence to justify the assumption that a very considerable degree of specificity of opsonins does exist, a specificity which it will be evident would in itself entirely explain the variations in the opsonic indices of one serum for different organisms. It is almost certain that the other two factors must, to a lesser extent, influence the results, but at the same time it is probable that the variations above referred to, are, in reality, largely due to the specific nature of opsonic action./

action.

In the third series of observations, it will be seen that the variations in the Opsonic Indices of a number of healthy individuals are somewhat greater than the majority of those on record, but taken all over, they bear out the general opinion, that in health the opsonic index may vary from .80 to 1.20.

The next series, namely those having reference to the effects of keeping sera under different conditions, were also undertaken with a view to obtaining some information on the question of the possible leucocytic origin of the opsonic content of the blood. Taken all over the results of these observations would seem to justify the assumption of some such origin. The results in themselves are of so definite and constant a character, that the question of experimental error can be safely excluded. That they are limited in number is admitted, but what they lack in quantity is, I think, to some extent made up for in consistency. To refer to these results again, we find the following averages:-

Average/

Av. Index of 16 sera kept in contact with the Corpuscles.	1.06
Av. " of 13 sera kept separate from the Corpuscles	.63
Av. " of 6 sera kept in open capsules and thus exposed to Bacterial contamination	.77
Av. " of 5 sera kept separate from the corpuscles.	.61
Av. " of the same sera incubated with fresh washed corpuscles for 24 hrs.	.72

The conclusions which I have arrived at from a study of these results are - that when serum is kept even for prolonged periods, the presence of the corpuscular elements not only preserves the opsonic power of that serum, but would also seem to increase it to a slight extent: that the opsonic power of serum is very considerably diminished, when it is kept for any length of time separate from the corpuscles: that even when kept in contact with the corpuscles Bacterial contamination results in a very considerable reduction in the opsonic power: that the opsonic power of a serum which has been kept separate from the corpuscles can be considerably increased by incubation with fresh washed corpuscles for 24 hrs. and that the amount of this increase is probably considerably greater than would appear/

appear from a consideration of the figures for two reasons, namely:- that the addition of an equal volume of fresh corpuscles suspended in saline solution dilutes the actual opsonic content of the original serum, and also because in these experiments adequate precautions were not taken to ensure that the washed corpuscles were entirely free from Bacterial contamination and so the addition of corpuscles so contaminated would necessarily reduce the opsonic content of the serum to a greater or lesser extent. Taking both these facts into consideration, it is likely that the actual increase is probably much greater than at first sight appears.

All these results taken together suggest that the corpuscular elements of the blood, must to some extent at least be concerned in the production of opsonin.

The next series of experiments were undertaken on the assumption that the question of the specificity of the opsonins of normal serum had not been previously investigated. In the great majority of the literature on the subject of opsonins, no mention is made of this question, and the only recorded results which I have been able to find, are those/

those of Bulloch and Western on the one hand, and on the other, those of Simon, Lamar and Bispham; to both of which reference has already been made. In effect the observations of Bulloch & Western point to a total and complete specificity of the opsonins of normal serum. Those of Simon, Lamar & Bispham, on the other hand, point to an entire absence of any such specificity. In another place several objections have been raised to the technique employed by Simon, Lamar & Bispham, but it must be admitted that these objections do not apply to the same extent in the case of the absorption experiments which were carried out in this connection. The results of my own observations might be said to occupy an intermediate position, in so far as the degree of specificity demonstrated by these is in no way a complete and total specificity, but at the same time the results are of a sufficiently definite and regular character to indicate clearly that at least a certain degree of specificity of the opsonins of normal serum does exist. If we now take the latter observations by themselves, we find that the opsonic power of a serum is considerably reduced for all organisms by digestion with one variety. In practically every case, however, the opsonic power of a serum so treated is lowered/

lowered to a very much greater extent for that organism with which it was previously digested. Similarly, where a double emulsion is added to the mixture of washed corpuscles, and inactivated serum, one of the organisms constituting such an emulsion being the same variety as that with which the serum was inactivated, the leucocytes show a selective preference for that organism with which the serum was not previously treated: in other words the opsonic power of the serum is lowered to a greater extent for the organism with which it was previously inactivated. Here again, however, the specificity is in no way complete, the opsonic power for the other organism being at the same time very materially reduced. I have not seen any other experiments similar to the latter, i.e. in which a double emulsion was used, recorded, and so these are perhaps of some interest in so far as they confirm the results obtained by the other procedure.

The Experiments carried out with the saline solution in which the opsonised Bacteria were washed are also of some interest, as showing that the Bacteria when digested with serum, remove more of the opsonin than is actually required to prepare them for/

for phagocytosis and that the excess can be recovered by washing the Bacteria so treated with normal saline solution. A similar result has been recorded by Dean. Whether the opsonin thus recovered has any degree of specificity for that organism from which it is obtained, is not definitely shown by the few experiments carried out in this connection.

Regarding the various observations made with opsonised Bacteria, nothing further need be said here, as they were only carried out as controls to demonstrate that the Bacteria had been in reality actively opsonised.

The successful cultivation of opsonised Staphylococci is of some little interest as showing that combination with the substance preparing them for phagocytosis in no way diminishes their vitality, as is shown by the copious growth resulting in 24 hrs.

The next series of experiments namely those carried out with animals, were without exception disappointing and much time was lost in consequence. Sufficient reference has already been made to these in another place, and so nothing further need be said here.

The/

The next question which falls to be discussed is that of the modified method of estimating the phagocytic power of the blood in health and disease.

It has already been stated that this method is, in effect, a simple modification of the original technique described by Leishman. By the addition of Sod.Citrate, clotting is entirely prevented, without apparently in any way modifying the various constituents of the Blood. The leucocytes are preserved in an active state and are sufficiently numerous in the resulting film to make counting a matter of the greatest ease. The simplicity of the process and the ease with which it can be carried out, are advantages over the ordinary procedure which in themselves, are a matter of some importance. The saving of time is not, perhaps, so great as would at first sight appear, where a large number of bloods have to be examined at one time, but when only one or two estimations have to be made the whole procedure can be readily carried out in the time required to merely collect the leucocytes when the older procedure is adopted. Taking into consideration all that has already been said - vide Part I. - regarding the laborious nature of the technique/

technique involved, and the fact that in individual cases it is necessary to make repeated estimations of the opsonic index, in order that the best results may be obtained, both from a diagnostic and therapeutic point of view, it will be evident that any modification of the existing methods whereby these processes may be simplified or time saved, even though only to a slight extent, becomes a matter of no little importance. That such a result can be obtained by the adoption of some such modification as that already referred to, as the Modified Method, will be evident from a perusal of the description given in another place and in actual practice, the ease and rapidity with which the whole procedure can be carried out are very readily appreciated. The whole process is little, if at all, more laborious than an ordinary blood count, involving estimations of both the Red Blood Corpuscles and leucocytes and in practice, provided an Incubator is at hand, can be just as readily carried out. Other points will be referred to later as having a direct bearing on the adoption of this method. Meantime, however, it is proposed to discuss briefly the various experiments which have been carried out with a view to establishing the accuracy of the method. The most likely line of investigation suggested/

suggested for this purpose, would seem to be a comparison of the results obtained by the modified method with those obtained by the ordinary procedure for the same blood. In reality, however, such a comparison though of considerable interest, does not necessarily furnish conclusive proof, provided a difference in the results should exist, as to which of the two methods is, in effect, the more accurate, since it is admitted that the limit of experimental error, even with the ordinary method, is of some magnitude. For this reason, it would seem better in the first instance, to consider the two methods on their own individual merits. When this is done, it is at once evident that the two methods though presenting many points in common at the same time present several very striking differences. Of the points in common, one might mention that with each procedure, the quantities of the various constituents are accurately measured by the same means, the Bacterial Emulsions are the same, the mixing is conducted in the same way, the incubation is similar, the spreading and staining of the resulting films identical, and in each case a similar control is carried out. From a practical standpoint, these points in common, comprise many of the possible sources of experimental/

experimental error. Another possible source of experimental error when the ordinary procedure is used, arises from the fact that when the serum to be examined is separated from the clot by centrifugalisation a slight degree of haemolysis not infrequently results, and also whilst in some cases the serum is found to be absolutely clear and transparent, in others it is cloudy and translucent. What the true significance of these differences really amounts to, has not, I think, been investigated as they crop up sporadically, and cannot be anticipated nor can any explanation, as a rule, be given for their occurrence. There would seem to be little possibility of experimental error associated with the washed corpuscles, as these are generally accepted to be quite an indifferent factor, still it is, I think, possible that the amount of centrifugalisation to which they are, of necessity, subjected, may to some extent at least, impair their activity.

When the modified method is adopted these possible sources of error are eliminated, and the only other source of fallacy is to be found in the presence of sodium Citrate in the resulting mixture possibly influencing the results. On analysis, the constituents/

constituents of the final mixtures in the case of the two methods are approximately as follows:-

ORDINARY METHOD.

Corpuscles & Bacteria suspended in Blood Serum diluted with saline solution.

MODIFIED METHOD.

Corpuscles & Bacteria suspended in Blood Plasma diluted with saline and Sod.Citrate Solution.

It will thus be seen that so far as the accuracy of the two methods is concerned, the only other point for consideration is the possible influence of the Sod.Citrate on the resulting phagocytosis and this is, perhaps of less moment than would at first sight appear, since in estimating the opsonic index of a blood, the control is subjected to exactly the same influence. However, with the object of eliminating this objection, a number of observations were made, in which sera were tested on the one hand without the addition of Sod.-Citrate, and on the other hand with this addition. The results of two typical experiments have been given in detail in another place, in which the indices obtained were:-

1.	Untreated Serum	1.15
	Citrated Serum	1.12
2.	Untreated Serum	1.13
	Citrated Serum	1.10

As these results have been frequently confirmed, there can be little doubt that the influence of the Sod.Citrate on the resulting phagocytosis when the Modified Method is employed may be entirely eliminated for all practical purposes.

That some degree of clotting may take place during incubation is an objection which one might perhaps anticipate: in actual practice, however, it does not exist, and in no case have I ever observed the slightest evidence of clotting when the mixture was finally blown out on the slide prior to spreading the film.

Having, therefore, disposed of these objections, no other, so far as I can see, can be applied to the Modified Method which is not equally applicable in the case of the ordinary procedure. So much for a comparison of the two methods on their individual merits, other points will be referred to later, but, meantime, it is proposed to discuss the results obtained for the same blood by the two methods. From what has already been said it will follow that where a difference to any considerable extent in the results obtained by the two methods exists, the reason for such difference cannot be ascribed to a technical difference, but rather must an explanation be sought for in some other direction. A consideration of the results/

results obtained for the same blood by both methods brings out the following points.

1. In the great majority of the cases the results correspond almost exactly.
2. In only two of the twelve cases is there any material difference; in the one only slight, in the other, however, very marked.

Of the cases which show corresponding results nothing need be said; the fact that the results obtained with two different procedures are so closely related, is in itself a strong argument in favour of the accuracy of both methods. Of the two indices in which there is a material difference, one is for *Staphiloc. Pyog. Aur.* the other for T.B. In the former an index of 1.08 is recorded by the Ordinary Method, and only .45 by the Modified Method. In the latter an index of .97 by the Ordinary and 1.14 by the Modified Method.

As has already been stated the difference in the first case cannot be explained in any other way than by the introduction in this particular estimation of some experimental inaccuracy of sufficient magnitude, to entirely vitiate the result. What this inaccuracy may have been, I cannot, of course, say, but/

but I am inclined to think that the pipette in which this particular mixture was incubated, must have been inadvertently removed from the incubator much sooner than it ought to have been. If we now take the second case, we find that the difference here is not of very great magnitude, being in reality only .17, a difference which comes quite within the limits of normal variation and almost within the limits of experimental error. Whether the difference is actually due to either of these causes, or to another, namely a difference in the individual leucocytes cannot be definitely stated. This is a question which will have to be discussed, but as several of the observations yet to be referred to, have a direct bearing on the subject, it will perhaps be better to postpone this discussion until each of these observations have been individually referred to. The next question which falls to be discussed, is one of some importance, because in many cases where it is desired to estimate the Opsonic Index, it is quite impossible to incubate the mixture immediately it is obtained from the patient. For this reason it is necessary to adopt some modified procedure whereby this difficulty can be circumvented. For this purpose, an equal volume of citrate solution alone is added to the blood on withdrawal, and these are immediately thoroughly/

thoroughly mixed in the ordinary way and drawn up into the pipette which is at once sealed and the mixture kept until it is convenient to make the final mixture with Bacteria. A series of observations to determine the accuracy of this procedure were carried out, the results of which have already been recorded. From the first it was evident that the keeping in this manner, in most cases, resulted in a somewhat diminished phagocytic activity, and it was therefore necessary that the control blood from which it was proposed to calculate the Index, should be withdrawn at the same time, and subjected to exactly the same conditions until the final mixture of Bacteria could be conveniently made. If this be done, the resulting Index is in no way influenced by the length of time the blood citrate mixture is kept, but is practically identical with the index obtained for the same blood where the Bacterial emulsion was added at the time of withdrawal, and the mixture immediately incubated.

Other results already recorded showing the extent to which the phagocytic power is reduced by keeping for different periods, bring out a point of some little interest, namely, that this reduction is almost nil when the emulsion employed is a comparatively thin one, only yielding an average of about 2 ingested cocci per leucocyte; if on the other hand a denser/

denser emulsion is employed, so that the resulting phagocytosis will show an average of about 6 or 8 ingested cocci per leucocyte, the reduction resulting from keeping the blood citrate mixture is of greater magnitude. Two possible explanations of this occurrence are suggested.

1. That, as a result of the keeping a quantitative, or more likely a qualitative change in the opsonic content takes place, so that only a smaller number of individual Bacteria can be opsonised, and thus where a dense emulsion is employed, a proportion of the Bacteria will remain unopsonised whereas, if a thinner emulsion is employed all the Bacteria will be individually opsonised.

2. That, as a result of the keeping, the activity of the leucocytes becomes diminished, so that when a dense emulsion is employed they are only capable of phagocytosing considerably fewer Bacteria than when freshly drawn from the general circulation: on the other hand, when a thin emulsion is employed so that only an average of about 2 Bacteria/

Bacteria per leucocyte have to be phagocytosed, the leucocytes are still sufficiently active to carry out this lesser task completely.

Either of these suggestions would, I think, satisfactorily explain this phenomenon, but the observations in this connection are too limited to permit of any definite opinion being stated.

Other two observations have been recorded as having some bearing on the accuracy of the method, but these have already been sufficiently referred to in another place.

From these preliminary experiments it may, I think, be inferred that for all practical purposes the one method is as accurate as the other.

Having, so to speak, cleared the ground by these preliminary observations, we are now in a position to consider the results which have been obtained in the case of a considerable number of normal and abnormal opsonic indices when these have been estimated by the modified method. The results of the normal indices have already been referred to in detail, and little further need be added here. That they/

they compare very favourably as far as regularity and consistency are concerned with the results recorded by other observers is apparent, since of the 47 Normal Indices estimated, only one is under .90, namely that of .74 for *Staphylococcus Pyog. Aur.* This low index I am inclined to regard as indicative of a lowered power of resistance to the *Staphylococcus* amounting to the abnormal. The blood in this instance was obtained from a medical student, and it was not observed at the time that there was any evidence of *Staphylococcal* infection. Still it is quite possible that such may have existed without attracting attention, but no definite information was obtained on this point at the time, as the result was not anticipated, and I have not again had an opportunity of interviewing the student in question.

That even mild forms of *Staphylococcal* infection may result in a lowering of the Opsonic Index for that organism, I have little doubt, in fact, throughout the whole course of this work, I have had a striking demonstration that such is the case, as my own Index for *Staphylococcus*, when compared with the great majority of the other Bloods examined for that organism is/

is distinctly a low index. This low index I cannot help associating with the fact, that I almost constantly suffer from small boils on the neck, which must, I think imply a lowered power of resistance on my part to Staphylococcal Infection. If one considers the frequent occurrence of this condition in men, it is not to be wondered at that, in the case of the Opsonic Indices of a number of apparently normal men for *Staphylococcus Pyog. Aur.*, the limits of variation are somewhat greater than for other organisms. A reference to the results recorded bears out this statement, for it will be seen that whereas with *B. Coli.* and *T.B.* the entire range of variation is .22 with *Staphylococcus*, on the other hand, even if the abnormally low index of .74 is excluded, the total range of variation is .35.

So much for the normal Opsonic Index, as estimated by the modified method. The next point which we have to consider has reference to the behaviour of the Opsonic Index, estimated in the same way, but in abnormal conditions. In this connection the bloods of 30 patients suffering from various forms of Tuberculous Infection have been examined. The cases were not selected, but taken quite indiscriminately, and comprise all degrees from the most acute and active to the most circumscribed and Chronic varieties of/

of the disease. The indices and a short account of each case have already been recorded in tabular form, in the order in which they were examined. Of the 30 cases, the first 14 were patients under treatment in the wards of the Royal Infirmary, the remainder were patients at the Convalescent Home, Corstorphine. In no case had tuberculin treatment been resorted to.

Of the 14 cases in the Royal Infirmary, 9 were very definitely acute and active, and accompanied by pyrexia, and marked constitutional disturbance; 5 were less acute, and in only 2 of these was there any pyrexia. Of the 16 cases at the Convalescent Home, with the exception of general debility and weakness, in a few cases only, none presented any constitutional disturbance. The tuberculo-opsonic indices of the first 14 cases have been arranged below in two columns according to the acuteness of the disease, the numbers in the left hand columns corresponding to the number of case in the original table page 141.

Actively/

ACTIVELY ACUTE.

NO. T.B.
INDEX

1. = .53

2. = .54

3. = .66

4. = .44

7. = .58

8. = .61

9. = .56

10. = .45

14. = .56

AVERAGE

FOR

9 CASES = .54

LESS ACUTE.

NO. T.B.
INDEX

5. = .71

8. = .67

11. = .74

12. = .55

13. = .59

AVERAGE

FOR

5 CASES = .65

It/

It is not proposed to again give the indices of the convalescent patients in detail as it will only be necessary to refer to one or two of these individually.

The average index for the 16 cases, it will be found on reference to the original table is .73.

It will thus be seen that according to Wright's classification of Tuberculous Infections, at least 9 cases, if not 12, belong to the acute class with constitutional disturbance. If we now consider the opsonic indices of the 9 cases which must undoubtedly be included in this class we find that they are in striking contrast to the indices recorded by others for similar cases. For the sake of comparison some of the indices recorded by other observers are again submitted.

WRIGHT	25 cases to show the effect of heat on the serum of tuberculous cases. For the unheated sera the lowest index = 1.00, the highest being 1.80.
ROSS	100 cases taken indiscriminately, of these only 11% below .80.
URWICK	54 cases all varieties, only 13 below .80.
GRACE CALVERT	Early and acute cases show high and low/

low indices indiscriminately.

It may be stated that the large majority of the cases in which a high index was recorded by these authors were acute and accompanied by constitutional disturbance.

It would therefore appear that where the ordinary method is employed, the tuberculo-opsoneic indices in acute cases of Tuberculosis are in the great majority of cases high, and that a low index is to be regarded as the exception, whereas a low index is consistently obtained when the modified method is employed, at least, so far as the number of such cases already examined are concerned.

Of the remaining 21 cases of tuberculous Infection which I have examined, a low index has been recorded by the modified method in every one without exception and of these, only two show an index above .80. It is not proposed to discuss each case individually, but rather to refer briefly in the first instance to the two cases with indices above .80, and in the second place, to compare the results as a whole, with those obtained for the acute cases.

The two cases in which the indices were above/

above .80 are - vide table IV Page 141.

Case 21 Index = .86. Case 30 Index = .82.

CASE 21 - Index .86. was that of a man who had his great toe and the head of the first metatarsal amputated for Tubercular Dactylitis 5 weeks previous to examination. The wound had been somewhat unhealthy, but at the time when the tuberculo-opsonic index was estimated had almost healed. The man was not particularly robust but there was no evidence of pulmonary or other Tuberculosis.

In this case, an index of .86 is not, I think, contrary to what one might reasonably have anticipated.

CASE 30 - Index .82 was that of a child aged 5 or 6 years, with old standing Empyema. Two operations had been performed, the latter consisting in the removal of portions of 2 ribs, and the abscess cavity had, as a result almost closed. It was still being drained by a small tube, but very little discharge was coming away. In other respects the child was remarkably well nourished and healthy and/

and there was no evidence of other tubercular mischief.

In this case, it is, I think, probable that a lower index would have been obtained had the blood been examined at an earlier stage in the course of the disease and that the index of .82 is in reality the result of a gradual increase in the tuberculo-opsonic content of the blood coincident with the steady progress towards recovery.

There is one other case which might be referred to separately - namely -

CASE 29 - Index .78, that of a girl aged 16 yrs. in which a diagnosis of Intra-cranial tumour had been arrived at. The symptoms in this case were very indefinite: paralysis was largely confined to the eye muscles and especially the external Rectus, but paresis of other ocular muscles also existed to a lesser extent. There was marked headache and some giddiness but no vomiting nor optic neuritis.

No definite opinion had been expressed/

expressed by those in charge of the case regarding the possible situation and nature of the tumour. Taking into consideration, however, the age of the patient and the indefinite nature of the symptoms the possibility of tubercular mischief is not, I think, improbable and it is therefore of especial interest that a tuberculo-opsoneic index of .78 should have been recorded in this case. The after history of this case will alone show whether this index is an accurate one, and it is therefore impossible to express any further opinion at present.

If we now compare the indices of the 21 more or less chronic or localised cases with those obtained for the 9 definitely acute cases it will be seen that a distinctly higher index is obtained with the more chronic cases. Of the 9 acute cases only 2 show an index above .60 and on the other hand only 3 of the more chronic an index below .60, and of these 3, at least two, but for the absence of pyrexia, might be included in the former class namely -

CASE/

CASE 12 - Index .55 was that of a man with advanced Genito-urinary tuberculosis. One of his testicles had been excised some time previously for tubercular disease, and he was, at the time when his blood was examined, extremely weak and emaciated.

CASE 13 - Index .59 was that of a woman suffering from both Pulmonary and Abdominal Tuberculosis. She had been under treatment for some weeks and the temperature had subsided.

Taking the 30 Indices as a whole, they might be said to diminish in a fairly constant manner according to the severity and acuteness of the disease. In conjunction with the 12 normal tuberculo-opsone indices in which the variations were from .99 to 1.14 these results as a whole are, I think, remarkable for their consistency and in striking contrast to the wide range of fluctuation observed in the indices of Tuberculous patients, and especially in acute cases where the ordinary method is employed. It is admitted that the observations in the case of the modified method are limited, but unfortunately this particular line of investigation/

investigation was only commenced quite recently and it has therefore been impossible to accumulate a larger number of results for inclusion in this work. In so far, however, as the results already accumulated are of a remarkably definite and consistent character it is, perhaps, justifiable to attempt to explain them on the assumption that they will meet with confirmation as a result of more extended observation. In what follows, I have built largely on this assumption, and it is, therefore, only fair that this should be clearly understood from the very first as it is quite possible that further investigation may not entirely bear out the results already obtained.

In seeking a satisfactory explanation of the difference in the tuberculo-opsonic indices dependent on the nature of the method employed, we must necessarily, in the first instance, consider the two methods themselves. This has already been done in another place and the conclusion then arrived at as a result of experimental evidence was to the effect that the individual methods were equally accurate so far as the technique of the two procedures was concerned and that any difference in the results/

results obtained was therefore to be sought for in some other direction. If this be done it is found that one difference which is in effect the vital difference in the two methods still remains - namely - the nature of the leucocytes employed.

In the case of the ordinary method these are uniformly obtained from a common source: in the case of the modified method they are in each estimation the native leucocytes of the blood under consideration.

As all other differences are of a vastly subordinate nature, one is therefore, I think, justified in ascribing to this fact the difference which would seem to exist in the results obtained by the two methods. This brings us at once to the all important question of whether or not the leucocyte is in reality the indifferent factor in phagocytosis which, on practically every hand, it is stated to be.

Recognising that it would be apparently impossible to explain the different results obtained by the two methods otherwise than by a difference in the nature of the leucocyte employed, I have revised the experimental evidence on record, relative to this question, and as a result, I find that it/

it is not perhaps so conclusive as general opinion would seem to indicate. Briefly it is as follows -

The experiments on which this opinion would seem to be based were first described by Wright and Douglas and subsequently the results obtained by these authors were confirmed by Bulloch and Aitken but apart from these two sources I have not been able to find any other recorded experiments of a confirmatory nature. It would thus seem that the opinion arrived at by Wright and Douglas and confirmed by Bulloch and Aitken has been accepted on every hand at least in this country, as one of these established facts which it is unnecessary to call in question. On the continent, however, it has not met with the same acceptance but is in direct contradiction to the opinion of the entire Metchnikoff school. Nor is the experimental evidence on which this opinion is based in any way conclusive. In a few cases where the leucocytes and serum were in each case obtained from a normal source the results were in no way altered by an interchange of leucocytes, on the other hand, however, where one of the bloods under consideration was that of an individual suffering from a definite Bacterial Infection a similar/

similar result was not in every case obtained. To bring this out clearly, I shall quote the experiment of Wright and Douglas which has appeared in more than one publication in support of the opinion that the leucocyte is an entirely indifferent factor. In this experiment the patient's blood is that of an individual suffering from a definite Tubercular Infection. The experiment is as follows:-

A.(1)

Av.no. ingested
Bacilli per leuc.

Patient's corpuscles	
Patient's serum	for 36 leucs. .66
Emulsion of T.B.	

A.(2)

A.E.W's corpuscles	
A.E.W's serum	for 32 leucs. 3.1
Emulsion of T.B.	

B.(1)

Patient's corpuscles	
A.E.W's serum	for 31 leucs. 2.1
Emulsion of T.B.	

B.(2)

A.E.W's corpuscles	
Patient's serum	for 30 leucs. 1.3
Emulsion of T.B.	

Wright's/

Wright's criticism is here quoted -

"It will be seen that the phagocytic effect obtained with the patient's white corpuscles (in A.1.) was (in B.1.) increased more than 3 fold in consequence of the replacement of their native serum by that of the control blood. The phagocytic effect obtained with the white corpuscles of the control blood (in A.2.) was (in B.2.) diminished in an almost corresponding degree (approximately about two and a half times) by the replacement of their native serum by that of the Patient."

So far as Wright's analysis of the above experiment goes, no fault can be found with it, but at the same time it might, I think, have been carried a little further. If we do this now, taking the figures as they stand then it must also be admitted that the substitution of A.E.W's corpuscles for those of the Patient, increases the phagocytic power of the Patient's serum from .66 per leucocyte to 1.3 per leucocyte, in other words, the phagocytic power is exactly doubled. That the difference in the actual figures in the experiment does not, perhaps, appear very great, is due to the fact that the/

the average number of ingested Bacteria per.leuc. is small even in the case of normal serum: had, however, a denser emulsion been used this difference would probably have been a great deal more evident. Still, the important fact from our present point of view, is this; that the substitution of normal leucocytes for the abnormal.doubles the phagocytic power of the abnormal serum. This difference is brought out in an even more striking manner if, from the above figures, the tuberculo-opsonic index of the Patient's serum is calculated.

In doing this, the index for A.1. is calculated from A.2. which is taken as unity. The index for B.2. is calculated from B.1. which is taken as unity. The following are the two indices for the patient's serum.

- | | | |
|--|-------|------|
| 1. When the patient's serum is tested
with patient's leucocytes - | Index | ·21. |
| 2. When the patient's serum is tested
with normal leucocytes - | Index | ·61. |

That this difference is a striking one, is at once manifest and the experiment therefore, taking everything into consideration, so far from proving conclusively that the leucocyte is an indifferent factor might rather, it seems to me, indicate that the leucocyte/

leucocyte is perhaps almost as important a factor as the serum.

In another experiment published by the same authors, the following results were obtained. In this case, the patient was suffering from a chronic Staphylococcal infection, and his opsonic index for that organism had been increased from less than half the normal to almost double the normal, by the therapeutic inoculations of a Staphylococcal Vaccine.

A (2)	AV. NO. INGESTED COCCI PER LEUC.	INDEX
Patient's Serum		
Patient's Leucs.	25.7	1.97
Suspension of Staphcoc.		
A (2)		
Normal Serum		
Normal Leucs.	13	1.00
Suspension of Staphcoc.		
E (1)		
Normal Serum		
Patient's Leucs.	13	1.00
Suspension of Staphcoc.		
E (2)		
Patient's Serum		
Normal Leucs.	28.2	2.17
Suspension of Staphcoc.		

Here again it will be seen that the substitution of normal for the abnormal leucocytes increases the/

the phagocytic power of the abnormal blood from 25.7 per leuc. to 28.2 per leuc. The difference in this case is not perhaps so marked as in the previous experiment, but at the same time, one must take into consideration that, in this case the abnormal blood had been very considerably altered by the inoculations of appropriate vaccines, with the result that a condition of successful immunisation had been to a certain extent established, and as we shall see later in the case of a blood of this description, the source of the leucocytes does not so materially influence the phagocytic power as in the case of an abnormal blood, in which no such condition of immunisation has been produced.

We must now turn to a consideration of the results obtained by Bulloch, and Aitken in experiments conducted on somewhat different lines to those of Wright and Douglas. In this case, the plan adopted consisted in testing one and the same serum with a variety of different samples of leucocytes subsequently testing the different sera with one and the same sample of leucocytes. A number of normal bloods were examined in this way, the results of which point conclusively to the indifference of the leucocyte. In another series, the bloods of three Lupus/

Lupus patients were examined in the same way, with the following results:-

						AV.NO. INGESTED T.B.PER LEUC.
1.	Normal Serum	+	T.B.Emulsion	+	Normal Leucs.	= 5.7
2.	"	+	"	+	Leucocytes of Lupus Patient	= 5.4
3.	"	+	"	+	"	= 5.2
4.	"	+	"	+	"	= 5.3
5.	Serum of Lu- pus Patient	+	"	+	Normal Leucs.	= 2.5
6.	"	+	"	+	"	= 2.4
7.	"	+	"	+	"	= 3.2

Expressing these results as
the Opsonic Indices we have:-

1.	1.00
2.	.94
3.	.90
4.	.93
5.	.43
6.	.43
7.	.56

At first sight, these results would seem to
prove/

prove very conclusively that the leucocyte is an indifferent factor. If, however, the series is subjected to a more careful analysis, it is at once evident that it is incomplete in so far as the phagocytic power of a Lupus serum has, in no case been estimated in conjunction with the leucocytes of the same Lupus blood which, it will be shown later is just the combination one would expect to show a difference. Taking both the results of Wright and Douglas, and Bulloch and Aitken together, it must be admitted that they are somewhat limited only comprising as they do, Normal bloods - a few Tubercular cases with uniformly low tuberculo-opsonic indices in some of which the observations are at best, incomplete, and one case in which a condition of successful immunisation had been established towards Staphylococcus Pyog.Aur. No results are recorded in the case of Acute Tuberculous infections with fluctuating or uniformly high tuberculo-opsonic indices. Taking everything into consideration, I cannot think that these experiments conclusively settle the question of whether the leucocyte is in reality an indifferent factor or not, and in the case of at least one of the experiments recorded by Wright and Douglas, there is as/

as much evidence against this conclusion as there is in favour of it. For these reasons, therefore, and taking into account the striking difference in the tuberculo-opsonic indices obtained by the two methods already referred to, I cannot but conclude that the leucocyte is in all probability by no means the indifferent factor it is usually represented to be.

That the rôle of the leucocyte may have a direct bearing on the differences in the tuberculo-opsonic indices of the 30 Tuberculous cases, which I have estimated by the Modified Method, as compared with the indices of similar cases recorded by others, and estimated by the ordinary procedure must be at once apparent, and I have little doubt in my own mind that the leucocytes are in effect, the true cause of this striking difference.

The question of which index is the more accurate representation of the phagocytic power of the blood, must next be considered. Before it will be possible to answer this question satisfactorily, it will be necessary to accumulate much more experimental evidence than is at present recorded, but at the same time, in so far as much of this evidence would seem to point in one direction, it is, I think possible/

possible to at least suggest an answer, largely, I admit based on the assumption that further observation will confirm the results already recorded, but at the same time one which would seem to satisfactorily explain the large majority, if not all of the established facts, relative to the process of phagocytosis. In attempting to answer this question satisfactorily, I have been led to formulate a theory regarding the possible nature of phagocytic action.

In formulating this theory, the following fundamental principles have been taken into consideration.

A. That Haemolytic and Bacteriolytic and probably also Bactericidal properties of Serum are due to the action of two distinct elements - namely

- (1) A thermolabile element known as "Complement" or "Addiment" or "Alexin".
- (2) A thermostable element known as "Immune body" or "Amboceptor" or "fixateur" or "la substance sensibilisatrice".

B. According to Metchnikoff, these elements (1) and (2) are the products of an intracellular secretion on the part of the phagocytes. They are essentially intracellular, and only set free by disintegration of the cells, this process/

process being called phagolysis. As a result of recent discoveries, Metchnikoff has, however been forced to admit that where a condition of Immunity is established "fixateur" i.e. immune body may be liberated without the occurrence of phagolysis, and may therefore be found free in the Blood Serum. Also that, when a condition of immunity is established, the fixateur (immune body) becomes greatly increased within the cell, either at the expense of, or without affecting the alexin (complement). Metchnikoff, however, still affirms that "alexin" i.e. Complement is only set free as a result of phagolysis.

- C. According to Ehrlich, both these elements, immune body and complement, are found free in the blood serum. They are probably of cellular origin, and closely allied to similar elements concerned in the ordinary processes of metabolism. That Complement is a normal constituent of all sera, and Immune body is developed in process of immunisation, and gradually increases at the expense of the complement.
- D. That both Immune body and complement can exist side by side in the serum without combining.

E./

E. That either complement or immune body is capable of combining with the Bacterium or red blood corpuscle as the case may be, but that the presence of all three elements is necessary for the production of the specific effect, namely in the one case, Bacteriolysis, and in the other Haemolysis.

If these fundamental principles are accepted, and there would seem no reason why they should not be, as a vast amount of Experimental evidence has already been accumulated in support of each of them, then the process of Phagocytosis can, I think be satisfactorily explained in an analogous manner. Whether or not, the elements concerned in Phagocytosis are identical with the other bodies, involves the question of the multiplicity of these elements. That the immune body is, in all probability a separate element can, I think be taken for granted as the specificity of these bodies, is now universally admitted; on the other hand, the question of the specificity of complements is one, on which opinion would seem to be largely divided.

Having, so to speak, cleared the ground by these preliminary observations, it is now proposed to apply/

apply the above principles to the process of Phagocytosis.

In doing so we assume:-

1. That the process of phagocytosis, like other allied processes, is dependent on the action of two separate elements, on the one hand a thermolabile element, the complement on the other hand a thermostable element, the immune body.
2. That these two elements are of leucocytic origin, and are found side by side in the body of the leucocyte, but are incapable of action, unless one element has become liberated from the cellular protoplasm.
3. That under normal conditions only the complement is found free in the serum.
4. In cases of Bacterial infection, or in process of immunisation, the immune body gradually increases, and can be demonstrated as existing free in the serum to a greater or lesser extent.
5. That the immune body increases at the expense of the complement, and as a result we find,
 - (a) In the body of the leucocyte, greatly increased immune body, complement either unaffected, as in the case of successful immunisation/

immunisation, or greatly diminished, as in the case of Bacterial Infections.

- (b) In the serum, immune body and complement exist side by side, the relative proportions of each depending upon the degree of Immunisation, or the severity or otherwise of the Bacterial infection.

If we now analyse these assumptions, we find that a very great deal of experimental evidence can be cited, which though not perhaps definitely proving, at least strongly supports the possibility of their existence. Thus in regard to the initial assumption that the process of phagocytosis may in effect be the result of the combined action of two separate elements, we have the evidence of Muir and Martin amongst others, that the opsonins of normal serum are definitely of the nature of complements, an opinion, which was arrived at, as the result of a series of experiments which in themselves would seem to be entirely conclusive.

Admitted, therefore, that the opsonins of normal serum are of the nature of complements, the strong/

strong presumption naturally follows that another element of the nature of an immune body may also be concerned in the process of phagocytosis. Nor have we far to seek for evidence in support of this presumption, since the presence of a body in every respect analogous to the immune bodies concerned in the processes of haemolysis and bacteriolysis can be clearly demonstrated in certain sera, as for example immune sera, and the sera of individuals suffering from a Bacterial infection, in most, if not all of which both elements can be demonstrated as existing side by side.

The fact that no element corresponding to an immune body has been found in normal serum cannot, I think be taken as proof that such is not a necessary factor in the process of phagocytosis. The possibility that such an element may exist in the body of the leucocyte, has not so far as I am aware been suggested as a possible explanation of the opsonic action as concerned in phagocytosis. That such may, however, be the case cannot I think be doubted from a study of the various allied processes already referred to, and the entire Metchnikoff School might be cited in support of this suggestion. In addition to this support, however, I have been led to the conclusion/

conclusion that the two elements of the nature of immune body and complement concerned in phagocytosis are most probably contained in the body of the leucocyte for the following reasons.

- (a) Where normal bloods are compared, the source of the leucocyte would seem to be an indifferent factor.
- (b) Where abnormal bloods are compared, the leucocyte would seem to be an essential factor in certain cases.
- (c) Where a number of tuberculo-opsone indices are estimated, the leucocytes of the individual cases being in each case employed (as in the Modified Method) the indices thus obtained are of a definite and consistent character.
- (d) When, on the other hand, normal leucocytes from a common source are in each case employed (as in the Ordinary Method) and only the individual sera of the tuberculous cases tested, the indices thus obtained show a wide range of variation.
- (e) That this variation corresponds somewhat closely to the range of variation observed with the heated sera of Tuberculous patients.

As I have not previously had occasion to quote/

quote the results obtained with the heated sera of tuberculous patients, I might perhaps do so here. In this connection it will suffice to quote the results of 25 cases recorded by Wright, in which the tuberculo-opsonic indices of the heated sera varied from .09 to 1.70, only 3 of which were below .30. With the unheated sera of the same cases, a high index was in every case obtained, the variation ranging from 1.00 to 1.80.

Now, as a result of the heating all the thermolabile elements i.e. the complements are destroyed, and therefore on the assumption that two elements are required in order that phagocytosis may take place, a fresh supply of complement must necessarily be obtained from another source. If so much be admitted, then it will be evident that the only possible source of this fresh supply of complement must be the leucocyte. It will, therefore, follow that where the complement in the leucocyte is either in excess of, or just sufficient to satisfy all the immune body in the serum, the resulting phagocytosis will correspond to the amount of immune body in the serum; if, however, the amount of complement in the leucocyte has been so far reduced that there is not a sufficient quantity to satisfy all the immune/

immune body in the serum, the resulting phagocytosis will correspond to the proportion of immune body, which the complement in the body of the leucocyte is capable of satisfying i.e. will be reduced in proportion to the diminution of the complement in the leucocyte.

The first case in which all the immune body in the serum can be complemented by the leucocyte is analogous to what takes place in a process of successful immunisation; the second case in which only a proportion of the immune body in the serum can be complemented by the leucocyte is analogous to what takes place in the abnormal process of Bacterial Infection.

Before considering the distribution of the several elements in the case of immune and abnormal bloods in which the free complement has not been destroyed by heat, it will be better in the first instance to enquire how these elements are distributed in the case of a normal healthy blood. For the sake of simplicity a diagrammatic representation is given.

NORMAL BLOOD.

LEUCOCYTE CONTAINS.	SERUM CONTAINS.
Normal quantity Immune body.	Normal free comple-
Normal quantity Complement.	ment.

When/

When Bacteria are added to this blood, the free complement in the serum becomes immediately attached to them, i.e. they are opsonised. If these opsonised Bacteria now come in contact with a leucocyte the immune body contained in the latter at once fixes the complement + Bacterium to the leucocyte and so phagocytosis takes place.

When, however, a normal serum is heated and the free complement thus destroyed, nothing remains free in the serum capable of uniting with the Bacteria and as the complement and immune body contained in the leucocyte are firmly anchored to the cell protoplasm no phagocytosis can possibly take place.

We must next consider what happens when the body is invaded by Bacteria. Two results are possible.

1. If these protective elements are sufficiently strong and the Bacteria not too numerous the latter are immediately phagocytosed or otherwise destroyed by the bactericidal actions of the serum and so nothing happens except perhaps that a step is taken in the direction of Immunisation towards the invading Bacterium.
2. If on the other hand the protective elements are in themselves insufficient to rid the body completely of the Bacteria the latter settle down in the tissues and produce their pathogenic effect.

It will thus be seen that in order that
Bacteria/

Bacteria may gain an entrance into the body and settle down there, they must be either in large numbers, or of unusual virulence or else we must presuppose an antecedent diminution in the protective elements.

If we now apply this to the process of phagocytosis it follows that the protective element which must here be diminished, is the complement as this is the only element concerned in the process of phagocytosis which exists free in the serum. This might be represented diagrammatically as follows -

BLOOD PREDISPOSED TO BACTERIAL INVASION.

LEUCOCYTE CONTAINS

SERUM CONTAINS.

Normal quantity Immune Body
diminished complement.

diminished comple-
ment.

As the organism settles down and multiplies the reserve forces of the body, so to speak, are called into play i.e. the machinery of immunisation is set in motion and as a result the immune body contained in the leucocyte becomes steadily increased, after a time a certain amount of the immune body is liberated from the leucocyte and is found free in the serum. As the immune body in the leucocyte increases it does so at the expense of the complement, and at the same time a progressive diminution in the free complement in the serum takes place. Such a diminution/

diminution in the complement may, I think be safely assumed in light of the many discoveries which have been made in the case of analogous processes involving the combined action of two similar elements.

Thus for example in connection with the preparation of Antibacterial sera it has been clearly proved that a serum may be abundantly rich in Immune body and yet exert very little, if any Bactericidal effect and it has been further proved that this failure in action is entirely due to the absence of an adequate quantity of complement and further that the action of such an antibacterial serum can be greatly increased if the deficient complement is supplemented by the addition of a normal serum in which a certain amount of free complement naturally exists. This fact would seem to have been proved beyond all possibility of doubt in the case of antibacterial sera and so I do not think it is too much to assume that a corresponding diminution in the complement concerned in the process of phagocytosis may take place as a direct result of a Bacterial invasion. If we represent this diagrammatically we find the following distribution of the various elements.

I.

When the Bacterial invasion is slight or results in a Chronic localised infection and when in consequence the machinery of immunisation is only stimulated to a slight extent -

LEUCOCYTE CONTAINS.	SERUM CONTAINS.
Increased Immune body diminished complement.	Small quantity Immune body considerably di- minished complement.

If Bacteria are now added to this blood the following sequence of events will take place. In the first place, the complement which still remains free in the serum will immediately attach itself to as many of the Bacteria as it can, and as a result the small quantity of Immune body which exists free in the serum will be immediately fixed by a corresponding quantity of the complement already attached to the Bacteria, and as a result we will have -

- (a) A certain proportion of Bacteria to which both immune body and complement are attached.
- (b) Bacteria to which only complement is attached.
- (c) Bacteria to which nothing is attached.

Of/

Of these (a) and (b) are actively opsonised
i.e. prepared for phagocytosis.

- (c) Are unprepared and cannot be phagocyted.
- (a) Are phagocyted by the leucocytes irrespective of the elements contained in the latter.
- (b) Are phagocyted as a result of the complement attached to them becoming fixed by the immune body contained in the leucocyte.
- (c) Are incapable of being phagocyted.

The degree of phagocytosis will thus be diminished whether the leucocytes employed in estimating this are obtained from the same source as the serum or from a normal blood.

II.

When the Bacterial invasion is marked or results in an acute or generalised lesion and where in consequence the machinery of immunisation is stimulated to a marked extent.

LEUCOCYTE CONTAINS.	SERUM CONTAINS.
Marked increase in Immune Body.	Considerable quantity of Immune Body (variable).
Marked diminution of complement.	Marked diminution of complement.

When/

When Bacteria are brought in contact with this blood as in estimating the phagocytic power, the following sequence of events will take place.

The small quantity of complement free in the serum at once attaches itself to as many of the Bacteria as it can. This complement will then fix a corresponding amount of the free Immune Body, and what still remains of the immune body becomes attached to as many of the Bacteria as it can, and as a result we will have. -

- (a) Bacteria to which both complement and immune body are attached.
- (b) Bacteria to which only immune body is attached.
- (c) Bacteria to which nothing is attached.

As before (a) and (b) are actively opsonised i.e. prepared for phagocytosis.

- (c) Are unprepared and cannot be phagocyted.
- (a) Are phagocyted by the leucocytes irrespective of the elements contained in the latter.
- (b) Are phagocyted as far as the diminished complement in the body of the leucocyte will permit. In other words as soon as the small quantity of complement remaining in the leucocyte is saturated with the immune bodies + Bacteria, no further phagocytosis can take place even although a considerable number of the remaining Bacteria are still actively opsonised by virtue of the immune body which has attached itself to them. The degree of phagocytosis is thus markedly diminished irrespective of the amount of immune body found/

found free in the serum, provided the leucocytes employed in estimating the phagocytic power are obtained from the same source as the serum and in which there is a marked deficiency in complement. The analogy between this result and that already referred to in the case of Anti-bacterial sera is very striking.

On the other hand if the leucocytes employed in estimating the phagocytic power of the serum are obtained from a normal source, and in which therefore no deficiency in complement exists, the resulting phagocytosis will be very markedly increased since all the immune body in the abnormal Serum to which Bacteria have become attached will now be readily fixed by the complement in the normal leucocyte and the Bacteria will in consequence be immediately phagocyted. It may be stated in passing that it is to this fact that I attribute the different results which I have obtained in estimating the tuberculo-opsonic indices of acute cases of Tuberculosis by means of the Modified Method in which both the leucocytes and serum are obtained from the patient as compared with the results recorded by others for similar cases but in which the tuberculo-opsonic indices were estimated by means of the ordinary method when the serum of the patient is in every case tested with normal leucocytes. In the case of the latter results/

results the high indices would correspond to cases in which there is a large quantity of free immune body in the serum and the fluctuations observed from day to day can be quite well explained as heretofore by assuming that an auto-inoculation of the general system from the seat of infection takes place. The direct result of such an auto-inoculation, is in the first place, to decrease the quantity of free immune body and probably also the free complement in the serum - the negative phase - subsequently, however, the immune body becomes again increased in excess - the positive phase -. When normal leucocytes are employed these fluctuations are readily detected as the normal leucocyte contains sufficient complement to satisfy all the immune body in the serum, when, however, the abnormal leucocyte is used these fluctuations cannot be detected as the small quantity of complement contained in the leucocyte is only sufficient to satisfy a small proportion of the total quantity of immune body in the serum.

The following observation confirms to some extent the suggestion regarding the probable incapability on the part of the abnormal leucocyte to take up more than a proportion of the immune body. The experiment was carried out some time before this theory/

theory in its present form was elaborated and at the time the results were not appreciated, but were simply attributed to a difference in the functional activity of the two leucocytes.

The experiment consisted in a comparison of the opsonic Index for *Staphylococcus* estimated on the one hand, by the Modified Method and on the other by the Ordinary Method, in the case of one of the two Guineapigs which I had attempted to immunise towards *Staphylococcus*. The Guineapig in question was Guineapig E. which as a result of the peritoneal injections had developed a considerable degree of Ascites. No injections of *Staphylococci* had been given for 12 days but a considerable degree of Ascites still persisted. The control was furnished by my own blood. -

RESULTS.	ORDINARY METHOD.	MODIFIED METHOD.
Self	1.00	1.00
Guineapig E.	.64	.21
Self Serum heated to 60°C. $\frac{1}{2}$ hour.	.05	
Guineapig E. Serum heated to 60°C. $\frac{1}{2}$ hr.	.40	

On analysis these figures are I think of particular interest and the point which at once strikes/

strikes one, is the fact that the heated Guineapig serum when tested with normal leucocytes shows a greater phagocytic power than does the unheated serum when tested with the abnormal leucocytes as in the case of the index estimated by the Modified Method.

A diagrammatic representation of the several elements in this blood would be as follows.

GUINEAPIG E.

LEUCOCYTE CONTAINS	SERUM CONTAINS.
Abundant Immune Body	Considerable quantity
practically no complement.	immune body.
	Very small quantity
	complement.

When the Bacteria are added to this blood the free complement in this case, a very small quantity becomes attached to as many of the Bacteria as it can, and this combination then fixes a corresponding quantity of the free immune body, the remainder of the immune body attaching itself to as many of the Bacteria as it can - thus we have -

- (a) Bacteria to which both immune body and complement are attached.
- (b) Bacteria to which only immune body is attached.
- (c) Bacteria to which nothing is attached.
- (a) and (b) are actively opsonised i.e. prepared for phagocytosis.

(c)/

- (c) Are unprepared and cannot be phagocyted.
- (a) Are immediately phagocyted by the leucocytes irrespective of the elements contained in the latter.
- (b) In this case practically none of the Bacteria to which only immune body is attached can be phagocyted as there is almost no complement in the leucocyte to which the immune body can become fixed. The resulting phagocytosis is therefore, very limited as is shown by the index of '21 obtained by the Modified Method.

If, however, normal leucocytes are substituted for the abnormal then (a) are phagocyted as before and in this case (b) are also phagocyted as there is plenty of complement in the normal leucocyte to which the immune body can become attached. The resulting phagocytosis is therefore, considerably increased as is shown by the index of '64 obtained by the Ordinary Method. As furnishing a concrete example of what has been laid down more or less on theoretical grounds these results are of the very greatest interest and apart from some such explanation I have not been able to imagine any other which would at all account for their occurrence.

There is just one other condition which we must consider, and that is the distribution of the several elements in the blood where a condition of successful immunisation has been established. In-diagramatic/

diagrammatic form this would be as follows.

IMMUNE BLOOD.

LEUCOCYTE CONTAINS.

Large amount Immune Body.
Complement somewhat di-
minished but not marked-
ly so or not at all.

SERUM CONTAINS.

Large quantity Immune
Body.
Complement diminished.

In this case when Bacteria are added to the blood, the free complement immediately attaches itself to as many of the Bacteria as it can, and this combination then fixes a corresponding quantity of free immune body the remainder of the immune body becoming attached to as many of the Bacteria as are left. We thus have.

- (a) Bacteria to which both immune body and complement are attached.
- (b) Bacteria to which only immune body is attached.
- (c) In this case there will be no bacteria to which nothing is attached but rather will there be a surplus of immune body, there being no bacteria left to which it can become attached.
- (a) and (b) Are as before actively opsonised i.e. prepared for phagocytosis.
- (a) Are immediately phagocyted by the leucocytes irrespective of the elements contained in the latter.
- (b) Are phagocyted by virtue of the complement contained in the leucocyte to which the immune/

immune body can become attached.

Such it would seem to me is the most likely distribution of the several elements in the case of an immune Blood. It would, therefore appear that just as in the case of antibacterial sera so also in the process of phagocytosis, the production of a true immune serum would seem to depend on the elaboration of a large quantity of immune body without at the same time diminishing the quantity of complement. This it will be seen is the essential difference between a true immune serum and a serum the result of a Bacterial infection and similarly in the case of the elements contained in the body of the leucocyte where the process of phagocytosis is under consideration. From this it will follow that in Bacterial Infections where recovery takes place a condition analogous to that of a true immune blood must be eventually established by the unaided efforts of the body.

If we now briefly recapitulate the various points which have been considered in discussing this theory we find.- that as a theory it explains the known facts regarding the opsonic action of -

1. Normal unheated serum.
2. Normal heated serum.
- 3/

3. Immune Serum unheated.
4. Immune serum heated.
5. Serum of a Patient suffering from a bacterial infection - acute or chronic more especially in the case of Tuberculous infections.
6. The heated sera of patients suffering from the same conditions.

In addition it would also seem to satisfactorily explain the following points.

7. That when the phagocytic power of normal bloods is compared the leucocyte would seem to be an indifferent factor.
8. That in the case of certain abnormal bloods especially the milder and more chronic forms of bacterial infection the leucocyte would also seem to be an indifferent factor where the phagocytic power of these bloods is compared with the normal.
9. That in cases where the Bacterial infection is more acute or more generalised the extent of the resulting phagocytosis would seem to be very largely dependent on whether the normal or the abnormal leucocyte is employed in estimating the phagocytic power.

In the case of the first 6 points the theory has been simply applied to explain certain facts which have been established by the experimental research of many authorities. In the case of the last three points, the experimental evidence is admittedly insufficient but at the same time, what evidence/

evidence there is points conclusively in the one direction and in addition several anomalous results can be explained in this way and apparently in no other way. The theory as such is also equally applicable to the various other established facts regarding opsonins, a summary of which is appended at the conclusion of Part I. (vide supra.)

The fundamental principles already referred to and on which this theory is based have been proved experimentally in the case of the corresponding elements concerned in the processes of Haemolysis and Bacteriolysis. Taken as a whole this theory is largely an application of that advanced by Ehrlich to explain these allied processes with this addition that a definite origin is allocated to the elements concerned, and in this connection Metchnikoff's hypothesis has been applied. The combination of these two theories is I think justified by Metchnikoff's own words which are to this effect that the two theories (referring to Ehrlich's and his own) may supplement each other but that they are in no way contradictory.

It is not now proposed to discuss the question of Vaccine treatment in relation to the possible/

possible explanation of opsonic action suggested in the above Theory; much more experimental evidence will require to be accumulated ere such a discussion is likely to prove of much value: but should such evidence support this view, there can be little doubt that it will have a very important bearing on the question of Vaccine inoculations.

Appendix

APPENDIX.

Since the completion of this work, I have been able to examine the tuberculo-opsonic indices of other 16 cases of Tuberculous Infection, and as these results form a valuable addition to those already recorded, I have thought it advisable to add them in the form of an appendix. As in the former case they comprise different varieties of the disease and in varying degrees of acuteness. In the subjoined table the most actively acute cases are again printed in red characters, the less acute and more localised cases in black. Two of the cases, namely Nos. 4 and 7, were examined on the first occasion as well, and are included in the original table, p.141, Nos. 4 and 14 respectively.

At the time of examination all the patients in the following Table were under treatment in the wards of the Royal Infirmary.

TABLE/

APPENDIX
to TABLE IV. (p. 141.)

NO.	NAME.	NATURE OF TUBERCULAR LESIONS.	T.B. INDEX.
1.	McCook.	Acute Active Pulmonary Tuberculosis.	.50
2.	Fenton.	Acute Active Pulmonary Tuberculosis.	.44
3.	McLeish.	Goitre with Tubercular Glands in Neck.	.82
4.	Tiffney.	Double Apical Phthisis Acute, active.	.44
5.	McManns.	Acute Active Pulmonary Tuberculosis.	.65
6.	Laidlaw.	Chronic Phthisis, slight Haemoptisis.	.71
7.	Sutherland.	Advanced Acute Pulmonary Tuberculosis.	.65
8.	Wilson.	Early Phthisis, slight temperature.	.72
9.	Harrison.	Acute Phthisis & Laryngeal Tuberculosis.	.66
10.	McLeod.	Tubercular Peritonitis with Pulmonary Phthisis.	.64
11.	Macrae.	Chronic Phthisis, no temperature.	.82
12.	Biggar.	Chronic Phthisis, no temperature.	.84.
13./			

APPENDIX (cont.)

NO.	NAME.	NATURE OF TUBERCULAR LESIONS.	T.B. INDEX.
13.	Chalmers.	Early Phthisis, no temperature.	.75
14.	Sinclair.	Early Phthisis Basal, no temperature.	.52
15.	Alexander.	Active Abdominal & Pulmonary Tuberculosis.	.63
16.	Cockburn.	Chronic Pulmonary Tuberculosis.	.85

These/

These results it will be seen correspond very closely with those already recorded in the previous table. Thus in every case a low index is obtained, and also the acute cases show a distinctly lower index than the more chronic varieties.

The averages are:-

- (a) For the 8 acute cases, .57 as compared with .54 previously obtained for similar cases.
- (b) For the 8 more chronic cases .75 as compared with .73 previously obtained for similar cases.

The Indices of the two cases examined on both occasions are:-

No.4	Ist Exam.	.44	2nd Exam.	.44
No.6	do.	.56	do	.65

Taken all over these indices are a little higher than those previously obtained, but not to any marked extent, and they again bear out the consistency shown in the previous series.

There is one other case to which reference must be made. It is not included in the Table as the diagnosis is somewhat uncertain.

The case in question is that of a man -
Stevenson/

Stevenson - aged 52, suffering from Pulmonary trouble. A diagnosis of Pulmonary Tuberculosis had been arrived at by those in charge of the case, and it was on this understanding that I examined his tuberculo-opsionic index. An index of 1.06 was recorded. I have since made a thorough enquiry regarding this case and as a result have elicited the following facts.

The Patient is a seaman and the present illness commenced acutely at the beginning of Feby. 1907, two days after a severe wetting while at sea. The onset was accompanied by shivering, the patient subsequently becoming very feverish. Cough also developed and caused him pain in the Right side. He remained feverish for some days, and was apparently acutely ill. Subsequently the fever disappeared but the patient did not regain strength, and the cough has persisted since that time. He has got steadily weaker and the cough has continued without abatement: throughout there has been a large quantity of thick dirty sputum. There have been no night sweats at any time, and there has been no temperature recorded above normal, since admission. There is no family history of tubercle.

The physical signs point to an apparently complete/

complete consolidation of the upper lobe of Right Lung the area of percussion dullness, marking off the borders of this lobe in a fairly definite manner. Coarse crepitation and Bronchial breathing are heard all over this area. At the Left apex, percussion is slightly hyper-resonant and expiration somewhat prolonged but there are no accompaniments: otherwise both lungs are apparently healthy, the physical signs in other regions being more or less normal. The sputum has been frequently examined, but no Tubercle Bacilli have been found. On the other hand I have since ascertained that the sputum is swarming with diplococci, quite indistinguishable from ordinary Pneumococci. The sputum is very thick and tenacious and does not in the least give me the impression of a tuberculous sputum; it is also brownish and apparently blood-stained in places.

This history is necessarily brief, but I think it will suffice to show that the diagnosis of Tubercle is by no means certain. Personally I am of opinion that the case is one of unresolved Pneumonia of the Right upper lobe, and the following points, I think, support this diagnosis.

(1) The age of Patient. (52)

(2) The acute onset of illness with shivering/
ing/

shivering and feverishness, following on a severe wetting whilst heated at work, and accompanied by cough and pain in side.

(3) The subsequent disappearance of fever with persistence of general debility and cough.

(4) The absence of night sweats throughout and of temperature since admission to hospital.

(5) The definite limitation of dullness apparently to one lobe.

(6) Absence of physical signs in other parts of lungs.

(7) Nature of Sputum, the absence of T.B., and the presence of large numbers of Pneumococci therein.

(8) Also there is no history of cough or lung trouble previous to present illness.

Taking everything into consideration, the tuberculo-opsonic index of 1.06 assumes a very significant character and I am not inclined to regard this index as an exception to the general rule that Tuberculous cases show a consistently low index, but the after history will alone afford conclusive proof as to whether it is in reality accurate or not.

Taken all over the indices of the 16 additional cases largely confirm the results previously obtained/

obtained for 30 cases, and for that reason they add very material support to the conclusions arrived at in the text which as already stated were largely based on the assumption that further investigation would be of a confirmatory nature.

Summary

SUMMARY of GENERAL CONCLUSIONS.

A Summary of conclusions regarding the opsonic content of serum, its nature, mode of action &c. arrived at from a study of the literature relative to this subject, has already been given at the conclusion of Part I. page 60.

The conclusions summarised below refer entirely to Parts II., III., & IV., and have been arrived at as a result of the various experiments carried out by the writer. They are as follow:-

1. That no definite parallelism can be demonstrated as existing between either the total number of leucocytes or the number of any one variety and the variations in the opsonic indices of normal individuals.
2. That one and the same normal serum may show a comparatively high opsonic index for one organism and at the same time a comparatively low index for another organism.
3. That this difference is in all probability due to a certain degree of specificity on the part/

part of the opsonins of normal serum.

4. That in health the opsonic index of normal individuals varies from about .30 to 1.20 in the case of *Staphylococcus Pyog. Aur.*, *Tubercle Bacillus*, *B. Coli* and *B. Typhosis*.
5. When the same blood is examined from day to day variations in the opsonic Index are also recorded, but the whole range of variation in this case is not very great, only being about .1.
6. That when sera are kept for varying periods, from 24 hrs to 18 days, in a sealed sterile glass capsule and in contact with the corpuscular elements, the opsonic power is completely retained and in the large majority of cases slightly increased, the average Index being 1.06 as compared with fresh normal serum.
7. That when the same sera are kept for the same time in the same manner but separate from the corpuscular elements, a marked diminution in the phagocytic power results, the average index being .63 as compared with fresh normal serum.
8. That where a serum such as that described in 7. is incubated with fresh washed corpuscles for/

for 24 hrs. the phagocytic power is again increased, the average index being .72 as compared with fresh normal serum.

9. That where sera are kept for varying periods in contact with the corpuscles, but in an open capsule and thus exposed to Bacterial contamination a marked diminution in the phagocytic power results, the average index being .77 as compared with fresh normal serum.
10. From the results referred to in 6, 7 and 8, I conclude that the corpuscular elements and of these most likely the leucocytes are in some way concerned in the production of opsonins.
11. That when a serum is digested for $\frac{1}{2}$ an hour with one variety of organism and the latter are subsequently removed by centrifugalisation, the phagocytic power of the serum so treated is thereby reduced to a considerable extent for all organisms but that the reduction is very much greater for that particular organism with which the serum has been previously digested.
12. That the same fact is brought out if the serum
so/

so treated is subsequently tested with an emulsion consisting of two varieties of organisms one of which being the same as that variety with which the serum was previously digested.

13. From the results referred to in 11 and 12, I conclude that a certain degree of specificity of the opsonins of normal serum probably exists but that this is by no means absolute.
14. That Bacteria when digested with normal serum remove from the latter more of the opsonins than are actually required to prepare them for phagocytosis and that this excess can be recovered by washing the Bacteria so treated in normal saline solution.
15. That Bacteria so treated and subsequently washed in normal saline solution are very actively opsonised and can be phagocyted by washed leucocytes in the entire absence of blood serum.
16. That opsonised staphylococci can be readily cultivated in the ordinary way yielding a copious growth in 24 hours.
17. That the intraperitoneal injection of a Bone Marrow emulsion in guineapigs alters to some extent the relative proportions of the different varieties of leucocytes, but that this has/

has apparently no definite effect on the opsonic Indices.

18. That the intraperitoneal injections, in guineapigs of an emulsion prepared from the mesenteric gland and spleen also alters to some extent the relative proportions of the different varieties of leucocytes but that this has apparently no definite effect on the opsonic indices.
19. That the intraperitoneal injections of a sterile staphylococcal emulsion in guineapigs in the first place diminishes the opsonic index for that organism subsequently however increasing it to some extent.
20. Although the opsonic index is thus increased only quite a small proportion of true immune body can be demonstrated as existing free in the serum.
21. In the case of one of the guineapigs, Ascites resulted from the intraperitoneal injections of Staphylococcal Emulsion and in this case the opsonic index for that organism assumed a very variable character, fluctuating within very wide limits.
22. That the opsonic index for B. Coli. remains unaffected by the intraperitoneal injections of a sterile Staphylococcal Emulsion.
23. That/

23. That the method described as the "Modified Method" is a simple and rapid procedure for estimating the phagocytic power of different bloods.
24. That the technique involved when the Modified Method is employed, introduces if anything fewer possible sources of experimental error than does the Ordinary Method.
25. That the addition of Sodium Citrate solution to the serum when the ordinary method is employed does not in any way alter the opsonic index of the serum under consideration.
26. That the presence therefore of the Sodium Citrate solution in the mixture when the Modified Method is employed does not in any way alter the opsonic index of the blood under consideration.
27. That the blood can be kept for a considerable time mixed with the Sodium Citrate solution and can be subsequently tested with bacteria without in any way altering the result, provided the control blood from which the index is calculated is subjected to exactly the same treatment.
28. That when the opsonic indices of the same normal bloods/

bloods are estimated by the modified method and by the ordinary method the results in the great majority of cases are practically identical.

29. From the results referred to in 24, 25, 26, 27, and 28, the modified method may be considered as accurate as the existing method.
30. That where the opsonic indices of a number of normal bloods are estimated by the modified method alone the range of variation in these is rather less than when the ordinary procedure is adopted.
31. That when a number of Tuberculous patients are examined the tuberculo-opsonic indices recorded by the modified method are of a very definite and consistent character.
32. That in the case of acute Tuberculous infections a very low tuberculo-opsonic index is invariably obtained by the modified method, a result which is in striking contrast to the tuberculo-opsonic indices of similar cases recorded by the ordinary procedure. In this connection however the results already accumulated are admittedly very limited.

33. From a general analysis of the tuberculo-opsonic indices estimated by the modified method for 30 tuberculous cases taken indiscriminately and comprising all varieties of the disease, the different indices would seem to diminish in a fairly definite manner according to the severity of the disease.
34. That the essential difference in the two methods namely the modified method and the ordinary method is - that in the case of the latter the leucocytes are in every estimation obtained from a common source whereas in the former the leucocytes are in each estimation those of the blood under consideration.
35. From a consideration of the above results and from a thorough revision of the experimental data on which was based the opinion that the leucocyte is an indifferent factor. I have been led to the conclusion that such is not the case, but rather that the leucocyte in certain cases, at least, is an essential factor in the process of phagocytosis.
36. In attempting to arrive at a satisfactory explanation of several of the results obtained in/

in the course of this work, a theory has been elaborated regarding the possible nature of phagocytic action.

37. This theory is based on several fundamental principles which have been established experimentally in the case of several allied processes.
38. As a theory it involves the combined action of two elements Complement and Immune body in order that phagocytosis may take place.
39. That both of these elements are contained in the cellular protoplasm of the leucocyte.
40. That only complement is found free in the serum under normal conditions.
41. That immune body is only found free in the serum when a condition of immunity is established or as a result of a bacterial Infection.
42. That under these circumstances immune body increases at the expense of the complement both in the body of the leucocyte and in the serum.
43. That this is entirely analogous to what has already been proved in the case of anti-bacterial sera in general.

44. That complement can exist side by side in the serum without combining.
45. That either complement or immune body is capable of uniting with the Bacterium.
46. That this combination immediately fixes a corresponding amount of the other free element.
47. That Bacteria thus combined with both immune body and complement are actively opsonised and can be phagocyted irrespective of the elements contained in the leucocyte.
48. That the Bacteria combined with complement alone are also opsonised but can only be phagocyted by virtue of the immune body contained in the leucocyte.
49. Similarly that Bacteria combined with immune body alone are also opsonised, but can only be phagocyted by virtue of the complement contained in the leucocyte.
50. That in the case of abnormal bloods the complement contained in the leucocyte is very considerably diminished and so the resulting phagocytosis is likewise diminished irrespective of the amount of free immune body contained in the serum.
51. When however a normal leucocyte is employed in estimating the phagocytic power this diminution/

diminution is not indicated as the normal leucocyte contains quite a sufficient quantity of complement to take up all the free immune body in the abnormal serum.

52. That the difference between a condition of true immunity and a condition the result of a bacterial infection is - that in the former case immune body is elaborated in considerable quantity, but at the same time the complement in the leucocyte is preserved in sufficient quantity to take up all the free immune body in the serum: in the latter case immune body may be elaborated in very considerable quantity, apparently depending on the acuteness of the Infection, but at the same time the complement in the leucocyte is so much diminished that it is incapable of taking up more than a small proportion of the free immune body in the serum.
53. That most, if not all of the known facts relative to opsonins which have already been established experimentally can be satisfactorily explained by this theory.
54. That in the same way some of the questions regarding/

regarding which diversity of opinion would at present seem to exist also meet with a satisfactory explanation.

Of the above conclusions those having reference to the theory regarding the possible nature of phagocytic action have been arrived at partly from a study of analogous processes, and partly as explanatory of several experimental results recorded in the course of this work.

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